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BEARING ON HEREDITY AND VARIATION

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ABSTRACTS OF PAPERS PREPARED FOR THE 1944 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

CLEVELAND, OHIO, SEPTEMBER 12–14, 1944

LAURENCE H. SNYDER, *Secretary*
Department of Zoology and Entomology
The Ohio State University
Columbus, Ohio

ABBE, E. C., and B. PHINNEY, University of Minnesota, Minneapolis, Minn.: *Interaction of genes for size and form in maize.*——The genes d_1, na_1, nl_2, and *py* have been introduced into the same inbred line (Minnesota Agricultural Experiment Station Inbred A188). Crosses have been made such that the following characters are segregating within the same progenies: d_1 and *py*, d_1 and nl_2, na_1 and nl_2. These double recessives may be recognized readily even in seedling plants. In every case they are smaller and grow more slowly than the single recessives. The characteristic features distinguishing each mutant reappear in modified form in the double recessives. For example, the stature of d_1d_1pypy is less than that of either of the single recessives. The leaf of the double recessive is shorter and relatively wider than the leaf of either single recessive, and much more so than in the normal. Since the size and form of the shoot apex is not significantly affected in these mutants, subsequent meristematic tissues are the seat of gene action and interaction.

BAUMGARTNER, I. N., Brown University, Providence, R. I.: *The inheritance of developmental patterns of rib ossification in the rabbit.*——A study of the rib ossification of 370 embryos of parental, hybrid and subsequent generations of two rabbit strains differing in rib number supplies evidence of a difference in time of onset of ossification governed by a single pair of genetic factors, a difference in rate of ossification controlled by at least several factors, and regional differences controlled by a number of factors. The rate of ossification shows two phases: an accelerated early phase, probably governed by a single pair of Mendelian factors, and a slow late phase which may be closely associated with general body growth. A theory based upon the response of rib formation in the two families to a determining substance at two thresholds is proposed as an explanation for the difference in time of onset. An alternative hypothesis suggests the independence of rate of differentiation and time of onset of rib formation. Variation and asymmetry were shown to decrease in amount with the decrease in ossification rate.

BLANC, RICHARD, Department of Biology, Amherst College, Amherst, Mass.: *The effect of temperature on compounds in the vestigial series of Drosophila melanogaster.*——Wing types derived from compounds of vg^{ni} (vestigial-nicked) and vg^{no} (vestigial-notched) with other vestigial alleles and deficiencies may be arranged in a number of intergrading series between normal and vestigial,

depending on the temperature level during development and on the type of compound. For compounds involving vg^{ni}, scalloping of the anterior margin of the wing was absent at 31°C, was present in a few wings at 25°C, and in most wings at 18°C, whereas compounds with vg^{no} always showed anterior scalloping. For both groups of compounds, destruction of the proximal posterior portion of the wing was more pronounced at 18°C than at 25°C, while notching of the distal portion was more pronounced at 25°C. However, the over-all destruction pattern was more extreme at 18°C for the vg^{ni} compounds, and at 25°C for the vg^{no} compounds. Results were least extreme at 31°C for both groups. Scalloping was generally more extreme among the females, but penetrance tended to be greater among the males for compounds in which not all wings were scalloped. That wing pattern depends on a relationship between temperature and time of development was shown by transferring vg^{ni} compounds from 32°C to 25°C or 18°C at different larval ages. The earlier the time of transfer, the more extreme were the types of scalloped wings. Whether this is the result of the lower temperature operating over longer periods of time or at earlier stages in development has not yet been determined.

BURNHAM, C. R., Univ. of Minnesota, St. Paul, Minn.: *Chromosome disjunction in maize interchanges.*——In plants heterozygous for interchanges involving chromosome 6 attached to the nucleolus, certain non-disjunctional and certain crossover spore quartet types may be recognized. The amount by which observed pollen abortion exceeds the amount predicted from the quartet study represents a non-disjunctional class which forms normal-appearing quartets but in which all four spores will abort. Such a study was made on plants heterozygous for T5-6c, in which about .2 of the long arm of chromosome 5 exchanged places with about .9 of the short arm of 6. Based on the noncrossover quartets, alternate or zigzag disjunction approximated 50 percent in all cases. Non-disjunction occurred exclusively or nearly so, as a result of separation along the axis of the "cross" carrying the two centromeres (plane-1 separation) with none or very few along the axis not carrying the centromeres (plane-2 separation). The former axis is also the longer one, the ratio of the two being about 8.3:1 at the pachytene stage. The same study was made on plants heterozygous for T5-6c but homozygous for an inversion in chromosome 5 which transferred that centromere to a position relatively close to the point of interchange. Non-disjunctional separation along plane-1 was reduced to about 32 percent while that along plane-2 was increased to about 20 percent. Thus, the centromere appears to have some effect on disjunction, since in this latter case the relative lengths of the two axes remained unchanged. When the inversion is heterozygous, the crossover results thus far seem to differ, depending on whether the inversion is in the interchanged or in the normal chromosome 5 and will require further study. Also available for study are two cases in which the short axis of the "cross" is the one carrying the two centromeres. When crossing-over occurs, homologous centromeres pass to opposite poles.

CHAPMAN, ARTHUR B., University of Wisconsin, Madison, Wisconsin: *Genetic and non-genetic sources of variation in the weight response of the immature*

rat ovary to a gonadotropic hormone.——The weight response of the immature rat ovary to an injected gonadotropin is commonly used as a means of assaying the gonad-stimulating potency of a preparation. Some of the sources of variability in this response of the ovaries of different rats of the same age to similar treatment have been identified and evaluated in a colony of rats in which there had not been any marked deviation from random mating. The mean ovary weight of the twenty-five-day old rats (N = 3257) which had been injected with a standard dose of an extract of pregnant mare serum was 67.5 mg. The standard deviation was 29 mg. Monthly changes in ovarian response due to unidentified non-genetic factors such as feed, health, crowding, general care of colony, batch of hormone used, etc. accounted for 15 percent of the variance. Intra-month autopsy body weight explained 16 percent more. After standardization for monthly fluctuations and variations in intra-month body weight the contributions to variance in ovary weight were approximately as follows: heredity of the individual, 35 percent; day-to-day, non-genetic, non-maternal influences, 15 percent; litter-to-litter postnatal effects, 10 percent; litter-to-litter prenatal effects and permanent maternal differences, negligible; unassigned or accidents of development, 40 percent.

CUMMINGS, JEAN M., Smith College, Northampton, Mass.: *Preliminary report on meiotic chromosomes in new Datura hybrids from embryos excised from incompatible crosses.*——The excising and culturing in vitro of embryos from incompatible parents not only provides a method of obtaining completely new plants, but also introduces the possibility of directly comparing the chromosomes of these species with those of the chosen standard line. This is important in analyzing the chromosomes. Before this it was necessary, by repeated backcrosses, to introduce the interchanged chromosomes into a bridging species before crossing them with the standard line. The chromosomes in the pollen mother cells of several of these hybrids are being studied. Configurations involving various numbers of chromosomes have been found. The chromosomes not involved in these configurations formed bivalents. For example, in the cross *Datura inoxia* × *Datura discolor* a configuration of 14 chromosomes involving a necktie was found. The other ten chromosomes formed five pairs of normal bivalents. Attraction of like ends, therefore, appears to be as common to these hybrids from incompatible crosses of Datura as they are to the compatible.

DEMEREC, M., and U. FANO, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Mutability to bacteriophage-resistance in Escherichia coli.*——In a strain of *E. coli*, changes to resistance to seven bacteriophages were studied. Among 377 mutants, eight different resistance groups were detected, Five of these occur with higher frequency than the other three. In addition, 364 mutants were derived from strains already resistant to one or more phages. The cross-resistance pattern of these mutants does not differ significantly from the pattern obtained when the mutants were derived from the original sensitive strain.—Results indicate that mutations to different resistance types are independent of one another and are probably produced

by changes comparable to gene mutations. It was found that mutants frequently show morphological differences in colony size, which breed true. At least three colony sizes can be distinguished for each of the eight types of mutants, and therefore at least 24 distinguishable mutant types showing some degree of resistance to our seven phages appeared in these experiments. Since many more phages affecting our strain could be isolated, it is evident that the actual number of mutants affecting resistance in considerably larger.

DUNNING, W. F., A. SEGALOFF, and M. R. CURTIS, Departments of Pathology and Anatomy, Wayne University College of Medicine, The Detroit Institute of Cancer Research, and The Woman's Field Army for Cancer Control: *Strain differences in response to diethylstilbestrol and the production of breast cancer in the rat.*——Significant differences in frequency of occurrence of pituitary adenomata, bladder papillomata and calculi, and breast cancer in three inbred strains of rats followed the implantation of pellets of diethylstilbestro Differences in mean adrenal and pituitary weights were observed eight weeks after daily graded injections of diethylstilbestrol in sesame oil.

EMMONS, C. W., and ALEXANDER, HOLLAENDER, Division of Infectious Diseases and Industrial Hygiene Research Laboratory, National Institute of Health, Bethesda, Md.: *Relation of ultraviolet induced mutants to speciation in Dermatophytes.*——The variability of certain dermatophytes (ringworm fungi) casts doubt upon the validity of current species delimitation. Mutants may appear spontaneously or following extraneous stimulation. As previously reported, exposure of conidia of *Trichophyton mentagrophytes* to monochromatic ultraviolet radiation induces mutation in young spores which have not yet shown spontaneous mutation. Several hundred induced mutants have been isolated and 40 selected representative strains have now been carried in subcultures for a period of five years without reversion to the original type or other observed change. Some of the induced mutants resemble other "species" (*T. granulosum*, *T. niveum*, *T. interdigitale*, *T. violaceum*). This emphasizes the close relationship within the group of dermatophytes and supports the hypothesis of a mutational origin for some of the "species" of dermatophytes.

GATES, WM. H., and MISS TERRY PULLIG, Louisiana State University, Baton Rouge, La.: *The linkage of dominant white spotting with hairless in the house mouse, Mus musculus.*——Roan (*Ww*) mice were tested for purity of the dominant spotting, and then crossed to hairless mice which in turn were genetically pure for self color. Both stocks were free of piebald. The F_1 was then backcrossed to the double recessive (*ww hrhr*) and the results noted. Since the stocks used were roan-haired and self-hairless, the records are of repulsion. Up to date 647 mice have been recorded of which 17 were albinos, and 25 died before identification. Of the 605 identified, 170 were roan and haired, 124 roan and hairless, 131 self and haired, and 180 self and hairless thus giving a crossover value of 42.1 percent $\pm$ 1.3 which is quite significant. Greater numbers are being raised to further check the results.

GAULDEN, MARY ESTHER, The Miller School of Biology and The Blandy Experimental Farm, University of Virginia: *An experimental study of somatic pairing.*——Previous investigators have not observed somatic pairing in *Crepis capillaris*. The present report deals with an attempt to induce it in this plant by expetimental means. The material used was from root tips of *Crepis capillaris*, 2n=6, each pair of chromosomes being morphologically distinguishable. In approaching the problem statistically, the chromosome arrangements, that is 3-, 2-, 1-, and 0-pair associations, were recorded for one thousand or more metaphases from each experiment. The data were tested with chi square to determine goodness of fit to a ratio of the arrangement frequencies calculated on the basis of chance. The control showed a good fit: P=.70—.50, thus no somatic pairing. All the pairs occurred in the same frequency. From root tips treated 96 hours at 1.5±1°C there was a tendency to pair, chi square yielding a P of less than .01. The C chromosomes were in a paired condition more frequently than the others. Pairing was also obtained in root tips subjected to 28–31°C for 24 hours: P=.05—.02. There was no differential behavior on the part of the individual chromosome pairs. It is suggested that there is a tendency for homologous chromosomes of *Crepis capillarsis* to pair somatically. At low temperature associations are facilitated by an increase in the length of prophase and a decrease in chromosome size in spite of a condition at this temperature which does not favor pairing. At high temperature some pairing is achieved in spite of a shorter prophase and an increase in chromosome size.

GILES, NORMAN H., JR., Department of Botany, Yale University, New Haven, Conn.: *Evolutionary patterns in the genus Cuthbertia.*——Cytological and morphological studies of extensive collections of Cuthbertia (a genus of the Commelinaceae related to Tradescantia) from throughout the range of the genus on the South Atlantic Coastal Plain of the United States, and analyses of species hybrids in the genus have made it possible to reconstruct in considerable cytogenetical, geographical, and chronological detail the recent evolutionary development of this group of plants. The pattern of evolution exhibited by this genus results principally from the combined effects of the following three processes: (1) Diploid speciation—three distinct species originally evolved from an ancestral diploid form as a result of isolation accompanied by the accumulation of differences due principally to gene mutations and in some degree to structural chromosomal rearrangements. Of these species *C. ornata* developed, apparently during early Pleistocene, following geographical isolation in middle Florida which was then an island; *C. rosea* became ecologically isolated on the mainland, principally in South Carolina and Georgia; and *C. graminea* developed with its major population concentration in the ancient Fall Line Sandhills of North and South Carolina. (2) Autopolyploidy—from the diploid *graminea* arose a vigorous autotetraploid form which spread onto the lower Coastal Plain terraces as the sea retreated during Pleistocene, eventually advancing into north-central Florida where it came into contact with the diploid *ornata* when the Florida island became permanently joined to the

mainland in middle to late Pleistocene. (3) Allopolyploidy hybridization of the autotetraploid *graminea* and the diploid *ornata*, followed by chromosome doubling in the hybrid, occurred giving rise to the newest element in the genus, an allohexaploid population in north-central peninsula Florida.

GOLDSCHMIDT, RICHARD B., Univ. of California, Berkeley, Calif: *The Blond-silver section of the X chromosome of Drosophila melanogaster.*——Between the scute section and the heterochromatic bulb a group of mutants is located which suppress pigmentation of body, hair or eyes in definite ways: Blond-translocation, silver and a number of specific suppressors. Deficiency tests locate all to the left of the *Bld*-break. Additional effects of some are pointed wings and trident. A series of new silver alleles combine the pale body of silver with pointed wings and suppression of speck and sable (not vermilion). The different phenotypic actions are present in different combinations in all the mutants of this section, suggesting allelism, but not of the standard, type as the different effects in compounds or with deficiencies further demonstrate. A better demonstration was obtained by combination effects with a second chromosome locus, bran. Bran plus the new silver alleles produces short blistered wings. Such a small recombination effect was found with *Bld* and *su-s* but a complete one with a small inversion with the left break in the *y*-sector (yellow phenotype) and the right one identical with the *Bld*-break, thus demonstrating position effects for both breaks. The salivaries of all alleles are normal to the left of *Bld*, but some alleles have very small rearrangements to the right. We conclude that all the bands between the *sc*-region and the bulb form a third section of the chromosome-tip in which all mutants or rearrangements produce a pigment bleaching effect of some kind.

GORDON, MYRON, New York Zoological Society, American Museum of Natural History, N. Y., N. Y.: *The XY sex-chromosomes determine maleness in wild populations of Platypoecilus maculatus while the WZ determine femaleness in domesticated breeds of the same species.*——For over twenty years fish geneticists (BELLAMY, GORDON, KOSSWIG, BRIEDER, etc.) have repeatedly found that domesticated female *Platypoecilus maculatus* are heterogametic (WZ) and the males are homogametic (ZZ) with respect to sex-linked genes. Further, FRASER and GORDON showed that W-borne dominant genes are transmitted from mother to daughters. In testing a wild population of *P. maculatus* from the Rio Jammapa at Plaza de Agua near Veracruz, Mexico, the opposite type of sex-determining mechanism was found: males were heterogametic and the females homogametic. A wild, spotted-sided (*Sp*) and spotted-dorsal-finned (*Sd*) male was mated to a striped (*Sr*), wild female: (Y)(*Sd*)/(X)*Sp*×(X)+/(X)*Sr*. They produced: (Y)*Sd*/(X)*Sr* and (Y)*Sd*/(X)+ males: (X)*Sp*/(X)*Sr* and (X)*Sp*/(X)+ females. *Sd* travelled from father to sons, *Sp* from father to daughters, and *Sr* from mother to both sons and daughters. A domesticated male mated with a wild female produced only male offspring: (Z)*Sp*/(Z)*Sb*×(X)+/(X)+=(Z)*Sp*/(X)+ and (Z)*Sb*/(X)+ sons in equal proportions. A wild female mated to one of the sons produced offspring similar in phenotype

and sex to their parents. Mating a wild male (Y)+/(X)+ to a domesticated female (W)*Sp*/(Z)*Sb* produced *Sp* daughters and *Sb* sons in equal proportions.

GORDON, MYRON, New York Zoological Society, American Museum of Natural Nistory, N. Y., N. Y.: *Similar sex-determining mechanisms in wild populations of Platypoecilus xiphidium, variatus and maculatus.*——From species crosses involving domesticated homogametic males with female *P. xiphidium* and *variatus*, BELLAMY and also KOSSWIG obtained all male hybrids. They concluded that *xiphidium* and *variatus* females were homogametic. The author working with native sex-linked genes in wild *P. xiphidium* from the Rio Purificacion and native genes in wild *P. variatus* from the Rio Tampoan, Mexico, confirms the conclusion that the males are heterogametic and the females are homogametic. Thus the three wild Platypoecilus species have the same sex-determining mechanism.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *An analysis of the genic or cytoplasmic basis of heterosis.*——Drosophila races of different geographical origin, formed by at least 20 generations of brother by sister mating, have egg productions ranging from 263–1606 eggs for their life average yields. The hybrid of 389×1000 egg races produced an average of 2034 eggs. The hybrids individually were not better than the best individuals of the inbred; simply all flies came nearer reaching this high production. This is the essence of hybrid vigor. Three experiments have been performed to analyze this better performance of these hybrids. Long inbred races with their similar genic and cytoplasmic constitutions have been contrasted with those made "homozygous" by out crossing—like genes as alleles but unlike cytoplasm. Crosses of inbred races have been analyzed to show the contribution of each particular race to (1) general high yielding ability of all hybrids with which it was crossed and (2) special ability to transmit high yield when crossed with particular races. Finally the contributions of each chromosome group to vigor have been analyzed by recombining the chromosomes comprising the inheritance, in all 27 possible ways. Since factorial designs were used, variations of genetic origin were properly segregated from those which were environmental. The results show that hybrid vigor was genetic in origin rather than due to cytoplasmic differences. Certain inbred strains contain genes which were generally effective in causing heterosis in their crosses. Other inbreds contained genes which were specific or limited in their effectiveness to particular inbred combinations. The redistribution of the total hybrid effect by chromosomes shows that it is due to a fairly large number of genes distributed at random over the chromosome pairs.

GREIFF, D., St. Louis University, St. Louis, Mo.: *The effect of zinc sulfate upon the fecundity and productivity of Drosophila melanogaster.*——Zinc sulfate was found in preliminary experiments to have a deleterious effect on productivity of *Drosophila melanogaster*. The following experiment was performed to determine the effect of various concentrations of the reagent on productivity

and to find in what period of the life cycle the greatest number of potential imagoes were eliminated. The controls were grown on Pearl's S-101 medium. The test media contained 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} grams of zinc sulfate per gram of control medium. The fifth and sixth sets failed to support the growth of yeast and were discarded. The productivity of the flies varied inversely as the concentration of the zinc sulfate present. The average number of flies per bottle was 173.7, 148.7, 121.2, 106.6 and 91.4. The unhatched eggs, the dead larvae, and non-emerged pupae were counted by means of a modified bacteriologic colony counter. There was no evidence that the number of potential reproductive units (p.r.u. = summation of undeveloped eggs, dead larvae, non-emerged pupae and imagoes) per mated day differed among the several series. Thus the females of each set laid approximately the same number of eggs per day. It was found that the number of undeveloped larvae exhibited the greatest increase with the increase in concentration of zinc sulfate. This was followed by the increase of unhatched pupae and unhatched eggs, respectively.

HOLLAENDER, ALEXANDER, and E. M. ZIMMER, Industrial Hygiene Research Laboratory, National Institute of Health, Bethesda, Maryland: *The effect of ultraviolet radiation and X-rays on mutation production in Penicillium notatum.*——Spore suspensions of *Penicillium notatum* were irradiated with monochromatic untraviolet radiation between 2280 and 2967 Å and medium hard X-rays. The mutation rate increased with increasing ultraviolet energy up to a maximum, and dropped with further irradiation. The most efficient wave length in the production of stable alterations was 2650 Å. Such results have been previously observed in *Trichophyton mentagrophytes*, *Aspergillus terreus* and *Neurospora crassa*. In the X-ray series the pattern of mutation production followed a linear relation with increasing energy. The highest (morphological) mutation rate obtained was about 40 percent of the surviving spores. More often a rate of 20 percent occurred at the peak of the curve in the ultraviolet. Different strains showed a varied response to ultraviolet. The morphological types of mutants will be demonstrated.

HOWARD, ALMA, and GERHARD SANDER, McGill University, Montreal, Canada: *Mother's milk and the age at which male and female mice diverge in body weight.*——The age at which male mice become heavier than female has been determined by weekly weighings: In strain C57 Black a significant difference first appears at four weeks; in strain *dba* at six weeks. Mice of each strain fostered on the other are similar in this respect to their foster mother's strain. The hybrid C57Bl ♀ × dba ♂ diverges at four weeks and the reciprocal hybrid at five weeks. These observations were made on samples of about 100 mice in each comparison. The heaviest and lightest mice of each sex were retained in each litter. Fosterings were made within twelve hours of birth.

The relevance of the observed influence of mother's milk on the relative growth rates of males and females to the question of maternal effect on growth rate in general will be discussed when results for other mouse lines have been analyzed.

HUNT, H. R., C. A. HOPPERT, and W. G. ERWIN, Michigan State College, East Lansing, Mich., and Henderson State Teachers College, Arkadelphia, Ark.: *Inheritance of susceptibility and resistance to caries in albino rats (Mus norvegicus.)*——The following diet will induce caries in the lower molars of albino rats: 66 percent (by weight) of coarsely ground polished rice, 30 percent of whole milk powder, 3 percent of alfalfa leaf meal, and 1 percent of sodium chloride. The purposes of this research were: (1) to determine whether there are hereditary differences in resistance to dental caries, and (2) if so, the number and nature of the gene pairs involved. Phenotypic selection, progeny testing of breeding pairs, and intensive inbreeding have been used for $7\frac{1}{2}$ years to build a caries susceptible and a caries resistant line. The rats were placed on the above diet when 35 days old, and the lower molar teeth have been examined fortnightly. Resistance was measured by the number of days on the caries diet required to develop a cavity. The phenotype has proved to be an unreliable basis for selection. The rats used numbered 3601. The mean of sibship averages in the susceptible line has steadily declined from 57 days (2nd generation) to 21 days (13th generation). The corresponding resistant mean has increased from 116 days (2nd generation) to 248 days (6th generation), 245 days (7th generation), and 216 days (8th generation).

HUSTED, L., and P. BURCH, University of Virginia and Radford State Teachers College: *The chromosomes of Virginia land snails.*——A cytological examination of 104 individuals of 17 species and subspecies in three genera of the family Polygyridae has shown: A uniform number and behavior of chromosomes between species and genera; a similarity in gross chromosome morphology; an heteromorphic bivalent in 38 percent of the individuals of *Triodopsis tridentata, T. t. justidens, T. t. edentilabris* and *Triodopsis fraudulenta* and a frequently encountered difference in chromosome number within the species *T. fraudulenta.* The heteromorphic bivalent is found in snails from Giles County and Patrick. It is similar in appearance wherever found. Fifty-three percent (17 out of 32) of the individuals of *T. fraudulenta* have 59, 60, 61, or 62 chromosomes rather than 58—the diploid number found elsewhere in the family. One snail is a mosaic with primary spermatocytes containing 62, 64, and 66 chromosomes. Those with extra chromosomes have been collected during two successive years and appear established on Doe Mountain (Giles County) at altitudes of 2500, 3300, and 4100 feet. The following species of the family have been examined: *Stenotrema stenotrema, S. hirsutum, S. monodon-aliciae, Mesodon thyroides, M. mitchellianus, M. andrewsae normalis, M. zaletus, M. appressus, M. rugeli, M. inflectus, Triodopsis dentifera, T. albolabris, T. tridentata, T. t. juxtidens, T. t. edentilabris, T. fraudulenta* and *T. t. vulgata.*

HUTCHINSON, J. B., R. A. SILOW, and S. G. STEPHENS, Cotton Research Station, Trinidad, B.W.I. (communicated by R. A. SILOW, The Blandy Experimental Farm—University of Virginia): *Evolution and domestication of cotton.* ——Genetical and cytological studies in Gossypium are sufficiently advanced to justify the preparation of a classification of the genus which, while being soundly based on data derived from them, will be acceptable to the taxonomist

as a reasonable and natural account of the species related to, and including, the cultivated cottons. The diploid species fall into three major groups between which chromosome homology is low. Members related to two of these groups, one confined to the Old World and the other to the New, have entered into the ancestry of the amphidiploid American cottons. Within main cytological groups progressive differentiation accompanying geographic divergence has led to the establishment of populations characterized by co-ordinated genetic systems harmoniously integrated to give a balanced genotype, but so different in gene content as to give rise to extensive polygenic segregation in crosses between them. Recombination products are less balanced than the parental forms, many being weak or partially sterile. Natural selection ensures their elimination. Populations whose integrity is maintained by such a genetic barrier are accorded specific rank. Just as their separation differs only in degree and not in kind from that between varieties, so also their distinction differs only in degree from that which exists between species which give highly sterile F_1 hybrids.—In the hairs which occur on the seeds of wild diploid species, both Old World and American, secondary deposition of cellulose in spiral pattern on the inner surface of the wall proceeds until the central lumen is almost completely obliterated. Domestication, which occurred in the Old World, was dependent upon a reduction in amount of secondary thickening, so that, on drying, the hairs collapse into flat convoluted ribbons which can be spun. It is reasonable to suppose that the New World amphidiploids acquired their lint from their Old World ancestor. The extensive cultivation of cotton, and its comparatively recent spread into areas subject to frost, has followed the development of the annual habit. This has occurred independently in all four cultivated species.

HUTT, F. B., Cornell University, Ithaca, New York: *Another breed of fowls characterized by a heterozygous phenotype.*——The distinguishing feature of the Erminette breed is the color of the plumage, which is predominantly white, with an admixture of enough black feathers to give a variegated appearance that is quite attractive. Feathers that are black, or partly so, are found in all regions of the plumage, but the proportion of these is less than in the somewhat similar pied plumage of the Exchequer Leghorn. The Erminette breed has been in existence at least forty-five years. Genetic analyses over a period of five years revealed that all typical Erminettes are heterozygous for an autosomal gene which induces pure white plumage in the homozygote. Erminettes mated *inter se* yield progeny of three colors in the ratio of 1 white: 2 Erminette: 1 black. Mating together the two undesired types (black×white) produces only typical Erminettes. Genetically the case is parallel with that of the Blue Andalusian. It constitutes the fourth breed in the fowl in which the distinguishing characteristic is found only in the heterozygote. Birds homozygous for the Erminette gene are indistinguishable from dominant whites and from recessive whites, which are genetically quite different. Similarly, although the plumage of the heterozygous Erminette superficially resembles the pied plum-

age of the Exchequer Leghorn, the two are genetically quite distinct, since the pied birds are homozygous for a recessive gene.

JAAP, R. GEORGE, Oklahoma Agricultural Experiment Station, Stillwater, Okla.: *Heritable intraembryonic hypertrophy of chicken testes.*——Natal testis weight has been observed to vary from 2.0 to 22.7 mg. Averages for male progeny from individual dams varied from 4.6 to 10.5 mg. Statistically, differences in natal testis weight were highly significant between progeny of varietal strains, of sires, and of dams within sires. Testis weight for the newly hatched chicks was not influenced by chick size and appeared to be free from seasonal influences. Crosses between large and small natal testis lines produced chicks with testes heavier than the average of the two parental strains. Differences in reciprocal crosses suggest that at least one gene for intraembryonic hypertrophy of the testes may be sex-linked. Whether the genes responsible for large natal testes are the same as those responsible for early sexual maturity of the female remains to be tested. Testis weight at hatching has been proved to be an indicator of potential body growth in the early post-natal period. This association is sufficient to make natal testis size an economical measure of the potential early growth of different strains within a variety. These observations demonstrate that the gonadotropic hormones are already being elaborated prior to hatching of the chick. It is probable that variation in testis hypertrophy may be attributable to either differences in gonadotropic stimulation or to responsiveness of the testis tissue.

KAUFMANN, BERWIND, and ALEXANDER HOLLAENDER, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Industrial Hygiene Research Laboratory, National Institute of Health, Bethesda, Md.: *Alteration of the frequency of X-ray-induced chromosomal breaks by use of ultraviolet and near infrared radiation.*——It appears possible, on theoretical grounds, to modify the capacity for reattachment of ends of chromosomes disrupted by ionizing radiation, if other types of supplementary treatment are given prior to the time that new combinations are established. Drosophila is well suited for experiments of this type, since the interval between irradiation of the spermatozoa and chromosome recombination may be prolonged for many days by withholding the males from copulation. An extended set of experiments designed to measure such alterations in the frequency of detectable chromosomal breaks has now been largely completed. Ultraviolet and near infrared radiations were used following administration of the total X-ray dosage, or in the interval between two fractional treatments. Determination of the frequency of breaks was made by analysis of salivary gland chromosomes.—Ultraviolet radiation of wave length 2537Å, following X-ray treatment, induces a reduction in the percentage of altered sperms and in the number of breaks as compared with the controls, which received only an equivalent dose of X-rays. Similar reductions in the percentages of altered sperms and of chromosome breaks were obtained in some experiments in which the X-ray treatment was

supplemented by near infrared radiation. The complete data cannot be presented here, but will form the basis of the demonstration, together with the designs of the special apparatus constructed for administering the ultraviolet and near infrared radiation.

KELLEN, LEONIE, Univ. of California, Berkeley, Calif. (introduced by R. B. GOLDSCHMIDT) *A sex controlled mutant of Drosophila melanogaster.*——Wavoid, a recessive wing character (2–40+), shows incomplete penetrance in the homozygous inbred stock and exhibits a marked sexual dimorphism in its manifestation. In the stock, the character appears in about 70 percent of the males and in 98 percent of the females. Some of the conditions which modify the penetrance of wavoid were determined. Most conspicuous was the effect of the addition of any Y chromosome to wavoid females (XX Y females) which reduced penetrance in the females to 70 percent. Homozygous yellow causes a more extreme decrease in penetrance than the extra Y chromosome, but it acts upon both sexes (females 22.5 percent penetrance, males 8 percent penetrance). Other mutants in the same region of the X chromosome have no modifying effect. High temperature (29°C), if applied to pupae younger than 168 hours (25°C) increases penetrance in both sexes.

KRUGELIS, E. J., Columbia University, New York City, N. Y.: *Alkaline phosphatase activity in the salivary gland chromosomes of Drosophila melanogaster.*——Alkaline phosphatase activity has been demonstrated in the chromosomes of the larval salivary gland of *Drosophila melanogaster*. The enzyme activity is detected by a cytochemical test based on the principle that phosphatase will hydrolyze phosphate-esters under controlled conditions. In the presence of calcium ions, insoluble calcium phosphate is precipitated at the site of enzyme action. Subsequent treatments with cobaltous nitrate and potassium sulfide yield a dark precipitate. The presence of the dark precipitate is considered a positive test for phosphatase activity. Comparison with the controls, in which no hydrolyzable phosphate-ester was added, shows a definite localization of the precipitate in the chromosome bands which correspond to the regions containing the greatest amount of nucleic acid. The ability of the alkaline phosphatase to split phosphate from adenosine nucleotide and the proximity of the enzyme to nucleic acid in the chromosome suggests a probable function in nucleic acid metabolism.

LEBEDEFF, G. A., Georgia Agricultural Experiment Station, Experiment, Georgia: *Inheritance of hard-shell in beans.*——The amount of hard seed in legumes varies with the environment. Evidence has been presented (LEBEDEFF, G. A., these Records, No. 11, 1942, and in press) indicating that hard-shell in beans is hereditary, but its expression is greatly affected by the moisture content of seed.—Out of 36 selections studied, two soft-shelled and four hard-shelled were intercrossed, and plants of these six selections as well as F_1 and F_2 plants from crosses between them were grown last winter in greenhouses at

Experiment, Ga. (the previous studies were made in Puerto Rico). The seed from these plants were kept in the laboratory for about three months, and then were put into an electric drier for five days prior to germination. The moisture content of the seed thus was reduced to about 6.6 percent.—When grown in Georgia the four hard-shelled selections became even harder, while the soft-shelled selections were unaffected. Under this environment hybrid seed from the crosses between soft-shelled and hard-shelled selections approached soft-shelled condition. Graphs are presented showing percentages of hard seed on successive days throughout the germination test in Parental selections and in various crosses between them. The experiment is still in progress.

LINDEGREN, C. C., Washington University, St. Louis, Mo.: *Heterokaryosis and heterosis in the fungi; their biological and industrial significance.*——Heterokaryosis is the natural condition of the filamentous ascomycetes. The natural heterokaryon in *Neurospora crassa* generally contains both plus and minus nuclei, but the plus and minus from the same locality are usually incompatible. Intra-strain sterility limits hybridization to matings between cultures from different localities. The genetical block to fertility can be removed either by anastomoses with another strain, or by diffusion of hormones (or vitamins) into the substrate. A homokaryon obtained from a freshly isolated mononucleate cell soon becomes heterokaryotic by mutation, and nearly all old cultures can be shown to contain a variety of genotypes. This condition generally reduces the potency of penicillin-producing cultures of *Penicillium notatum*. One artificially produced heterokaryon of *Penicillium notatum* was found to have a capacity for penicillin production much below that of either original culture. Another heterokaryon between an average- and a high-producer was found to have the same potency as the high producer, thus showing that the low-producer was deficient in some substance essential for penicillin production which the high-producer supplied to the combination in excess.

LURIA, S. E., Indiana University, Bloomington, Indiana: *Mutations of bacterial viruses and of their bacterial hosts.*——Bacterial viruses (Bacteriophages) can give mutants active on bacterial strains which, by mutation, have become completely resistant to the original viruses. A mutation of the virus compensates for a mutation of the bacterial host. Each mutant virus is indistinguishable from the parent virus serologically and in its action on the common host; it is, however, slowly adsorbed by its new host, which does not adsorb the parent virus. Since the virus mutation makes the virus adsorbable by the resistant bacteria, thus compensating for the bacterial mutation, it seems likely that the structural changes produced by both mutations are small, probably limited to simple stereochemical rearrangements. The mutations suppress or restore the complementarity of the surface structures that conditions the adsorption of a virus by the receptors of the bacterial surface.—A study of the bacterial mutants resistant to normal and mutant viruses showed the following relations: (1) Resistance to the parent virus may be caused in

the same strain by different mutations which may or may not lead to resistance to the mutant virus. (2) Bacterial mutations leading to resistance to a mutant virus also lead to resistance to the parent virus. (3) Bacterial mutations leading to resistance to unrelated viruses are generally independent: the same mutation to resistance to a virus can occur in strains which, because of previous mutations, differ in sensitivity to an unrelated virus.

McFADDEN, E. S., and E. R. SEARS, U. S. Department of Agriculture, College Station, Texas, and Columbia, Missouri: *The artificial synthesis of Triticum spelta.*——Through colchicine treatment an amphidiploid has been produced of the wild tetraploid wheat, *Triticum dicoccoides* (n=14), and *Aegilops squarrosa* (n=7). This amphidiploid is cytologically stable, highly fertile, and morphologically almost identical with an awned variety of *T. spelta* (n=21). F_1 hybrids of the amphidiploid with *T. spelta* (and *T. vulgare*) are of fairly regular cytological behavior, with 21 bivalents in about half the meiotic metaphases, and with only rare multivalents. Their fertility under favorable environmental conditions is almost complete. F_2's with *T. spelta* are uniformly fertile and exhibit segregation for minor characters only, with little tendency toward transgressive segregation or other phenomena usually associated with wide crosses. Thus *Ae. squarrosa* appears to be the source of the third set of seven chromosomes (the so-called C genome) of *T. spelta* and *T. vulgare.* Further evidence of this has been obtained by synthesizing *Ae. cylindrica* (n=14). An amphidiploid produced from *Ae. caudata* (n=7)×*Ae. squarrosa* has proved very similar in morphology and in chromosome constitution to *Ae. cylindrica*, which had previously been known to possess both the C genome and the genome of *Ae. caudata.* Identification of *Ae. squarrosa* as the possessor of the C genome should facilitate the transfer of characters from the tetraploid wheats to the hexaploid *T. vulgare*, by providing a means of avoiding the sterility normally encountered in the hexaploid-tetraploid hybrids. Following the addition of the *Ae. squarrosa* genome to the tetraploids, the resulting amphidiploids should yield highly fertile hybrids with *T. vulgare.*

McLEAN, SUZANNE W., Smith College, Northampton, Mass.: *New hybrids with the aberrant species, Datura ceratocaula, secured by embryo dissection.*—— *Datura ceratocaula*, an aberrant species in the genus Datura, is distinguished by characters adapting it to life in shallow ponds. Under usual conditions only about 0.1 percent of its seeds germinate. By culturing excised embryos, nearly perfect germination may be secured. Earlier extensive attempts to secure viable hybrid seed by crossing *D. ceratocaula* with the other nine herbaceous species of Datura were entirely unsuccessful. Hybrids may be secured, however, from the majority of crosses with other Daturas by culturing excised embryos. Eighteen species combinations are theoretically possible if *D. ceratocaula* is used both as male and female. So far eleven of fourteen crosses attempted have yielded dissectable embryos at different stages from undifferentiated to completely differentiated, though malformed, bodies. Eight hybrids have grown, three well enough to be planted into soil, of which two have grown

to maturity. These new hybrid embryos are important for the interpretation of chromosomal structure of species whose chromosomes have not yet been determined. In the hybrid with *D. inoxia*, the characters from *D. ceratocaula*,—whorled branching, puckering of leaf, appressed hairs on ventral leaf surface, and purplish corolla—were dominant. Leaf size, shape, and teeth on margin were intermediate, while yellow color of stigma and lack of spines on capsule were recessive.

MICKEY, GEORGE H., Louisiana State University, Baton Rouge, La.: *Synapsis and behavior of chromosomes in polyploid male germ cells of Romalea microptera, Beauv.*——Polyploid spermatogonia may undergo regular equational division and thus continue the same degree of ploidy. In synaptatene the autosomal homologues always synapse in pairs producing only bivalents; no trivalents, quadrivalents or other multiples have been observed. The X chromosomes, of which there are two in a tetraploid cell, persist as univalents rather than synapsing to form bivalents. As a consequence of such uniform behavior during synapsis, regular disjunction results from meiosis and spermatids are formed which contain complete sets of chromosomes (euploidy). These polyploid spermatids undergo all the typical changes during metamorphosis to produce giant sperms, which, however, have multiple centrioles and axial filaments.

MULLER, H. J., Amherst College, Amherst, Mass.: *The non-equivalence of the blocks and the salivary "heterochromatin."*——The view that different chromosome regions, whether "euchromatic" or "heterochromatic," have similar breakabilities, approximately proportionate to their mitotic length, was disproved by comparing the results of irradiation of two contrasting X chromosomes. One of these, the crossover scute 8-scute 4, was twice as large as the other, scute 4-scute 8, because of its containing two block-A units, which the latter lacked. These differing chromosomes gave sensibly the same frequency of translocations, and also of rearrangements visibly affecting genes of the left end. These results corroborate the conception of the blocks as being virtually unbreakable units. The fact that "heterochromatic" regions which include blocks ordinarily have a breakability approximately proportionate to the length of these blocks must be only a coincidence, caused by an inordinately high breakability of the "other heterochromatin," that exclusive of the blocks; as compared with euchromatin. That this high breakability is not an indirect effect of the blocks upon the remaining "heterochromatin" (caused for instance by some strain induced by the former, or by a transmission of radiation hits from them) is further shown by the above results, since chromatin lacking blocks has an equally high breakability. For given lengths of "resting" (salivary) chromatin, the breakability must be at least 30 times as high in this remaining "heterochromatin" as in euchromatin. What is called "heterochromatin" in mitotic chromosomes is almost exclusively blocks, whereas "heterochromatin" in salivaries is almost exclusively the remaining portion. It is misleading to identify two types of structure differing so essentially by giving them the same name, "heterochromatin."

OAKBERG, EUGENE F. and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *On the physical basis for genetic resistance of mice to mouse typhoid.*—— In earlier work we showed that the leucocyte numbers in the blood were significant elements differentiating resistant from susceptible mice. In this work the bases for these resistance differences are broadened to include significant changes in liver and spleen. Six strains of mice ranging from extremely susceptible to highly resistant are available for this study. The percentages of survival when mice of the particular strains are inoculated with 200,000 organisms of *Salmonella typhimurium* are Ba 8, L 13, E 53, Z 58, RI 75, S 86. The distributions of the liver lesions show that they progress rapidly in the most susceptible mice, Ba and L. In these strains the lesions seldom progress beyond pin head size without death occurring. Lesions of the more resistant mice, RI and S, progress more slowly and, on the average, become much larger, with the formation of yellow white areas of focal caseous necrosis. In the spleen the conditions are reversed. The Ba and L develop marked lesions and succumb rapidly to the disease. The resistant strains show few or no spleen lesions. Wide differences in organ reactions to typhoid are strain characteristics. The necrotic changes of the cells bear an intimate relation to the genetic capacity of the strains to resist the disease when once attacked. These differences in the physiological potentialities of the cells form part of the closely coordinated system which makes for genetic resistance to disease.

OLIVER, C. P., University of Minnesota, Minneapolis: *Four lozenge alleles phenotypically alike which react differently with their other alleles.*——Four of ten lozenge alleles in *Drosophila melanogaster* have eyes characteristic of lz^s (spectacle). The eyes are glassy-smooth, almond-ovoid in shape, although lz^{36} has a narrower ventral portion, and light-brown in color with sparse red-stippling over the eyes and a denser marginal rim; lz^{36} has less and lz^3 more total pigment in the central portion. All compounds of the four resemble lz^s except that lz^3 influences pigment density, and in lz^s/lz^{36} the narrower eye of lz^{36} dominates. The four in compounds with the other six alleles show a variety of reactions which suggest that the similar phenotypes may be due to different developmental reactions. This is especially true of lz^s and lz^{sB} which are indistinguishable phenotypically. As examples, lz/lz^{sB}, lz/lz^{36} and lz/lz^3 have wettish, pitted, almost glassy eyes, shaped like lz^s and with a color intermediate between lz and lz^s; but lz^s/lz resembles lz^{37} which is normal except for a small area of rough facets in the posterior portion of the eye. The compound lz/lz^{37} cannot be distinguished from lz^{37} males, a condition also found when lz^{37} is compounded with the non-spectacle-like alleles. However, the eyes of compounds of lz^{37} and lz^{sB}, lz^{36} or lz^3 are wettish and glossy, similar to lz^g; those of lz^{37} with lz^{sB} or lz^{36} resemble the slightly narrowed (oval) eye of lz^g but lz^{37}/lz^3 eyes are almond, similar to lz; and lz^{sB}/lz^{37} eyes are normal in color, lz^3/lz^{37} resemble extreme lz, and lz^{36}/lz^{37} have a blood color similar to lz^g. The significance with respect to genic action will be discussed.

OWEN, R. D., C. STORMONT, and M. R. IRWIN, University of Wisconsin, Madison, Wis.: *Genetic differences between two breeds of dairy cattle as shown by*

incidence of cellular antigens.——The frequency of each of 30 cellular antigens in a sample of 1305 Guernsey cattle was compared with that in 875 Holstein-Friesians. Evidence has been published that each of these antigens is gene-determined, but the genetic relationships among many of them are not yet clear enough to permit the calculation of simple gene frequencies. The antigen frequency in Guernseys ranged from 3.3 percent having $\bar{U}$ to 86.5 percent having $\bar{A}$, and in Holsteins from 1 percent having $\overline{P}$ to 78.2 percent having $\overline{H'}$. Seventeen of the antigens were significantly more frequent in Guernseys than in Holsteins; eight were more frequent in Holsteins (three of these lie between the .01 and .05 levels of significance); five were not significantly different in frequency in the two breeds. Each of the antigens was encountered in each breed. The breed differences in "blood phenotype" therefore appear to rest for the most part on contrasting frequencies of genes common to the two breeds, rather than on the fixation of alternative alleles in different populations. Possible parallels to other genetically determined race differences are obvious.

POULSON, D. F., Yale University, New Haven, Conn.: *Somatic mosaics and the differentiation of imaginal discs in Drosophila.*——Somatic elimination of the covering duplication in a stock of *Drosophila melanogaster* otherwise homozygous for lethal-7 ($Dp(1:f)X^{C^2}/l(1)7/l(1)7$) produces mosaic individuals which constitute about 5 percent of the adult progeny. The regions in which the duplication is lost are usually small, limited chiefly to the compound eyes, and are apricot-like in appearance. Occasional mosaics appear in which other portions of the head are involved. These show the pale yellowish coloration of cuticle and bristles to be expected on the basis of the appearance of the inter-ommatidial hairs in lethal-7 patches. A number of these mosaics are of interest in that they give support to recent work on the delimitation of the regions of the cephalic imaginal discs. One in particular (affecting the left eye and top of head) in which the dividing line between the two types of tissue passes through the median ocellus, clearly indicates that the latter is the result of fusion of separate rudiments derived from each of the two optic discs. In none of the cases observed are the antennae or proboscis involved, although embryological studies on normal stocks show that the cephalic imaginal discs are all laid down within a relatively short period. Mosaic patches in the thorax and abdomen are either very rare or lacking completely. Fairly high frequencies of abnormal tergites and uni-lateral wing deformities suggest cell-lethality in regions posterior to the head. The lack of abnormal or defective antennae and proboscides indicates complete lethality, non-autonomy, or regulation in these cases.

RILEY, HERBERT P., University of Kentucky, Lexington, Ky.: *Chromosome studies in some species and hybrids of Gasteria.*——Chromosome number was typical (four long and three small pairs), abnormalities at meiosis and the microspore division few, and pollen fertility high in *G. acinacifolia*, *Armstrongii laetepunctata*, *nigricans*, *nigricans* var. *fasciata*, *nitida*, *obtusifolia*, *planifolia*, and *sulcata;* pollen sterility was about 25 percent in *brevifolia*, *brevifolia* ×

nigricans, *brevifolia* × *planifolia*, and *planifolia* × *nigricans*, and about 50 percent in *pariviflora* and *pulchra* × *planifolia*. About ten percent of the microsporocytes and microspores were abnormal and about 40 percent of the pollen was bad in *verrucosa* × *brevifolia*. In a *sulcata* × *nigricans* triploid hybrid the microspores had from four to eight large and two to six small chromosomes, and about half the pollen was bad.

RILEY, HERBERT P., University of Kentucky, Lexington, Ky.: *The frequency of plants with small chromosomes in three populations of Tradescantia paludosa.*——Plants with very small supernumerary chromosomes have been known in Tradescantia for some time. The number of plants with none, one, two, three, and four, respectively, in three populations of *T. paludosa* are: (1) from Waggoner, thirty-six, two, seven, three, none; (2) from Micheaud, twenty-two, nine, eleven, six, none; (3) from the type locality at the intersection of the road to Chalmette, twenty-three, six, nine, five, and one. The last two localities are three miles apart; the first is twenty-two miles from the other two and is on the opposite side of the Mississippi River.

RISMON, GEORGE C., University of Pennsylvania, Philadelphia, Pa.: *Bimodal distribution of cell size measurements in Habrobracon.*——The frequent occurrence of platykurtic distributions in measurements of cell size suggested an experiment to determine whether a bimodal distribution of haploid individuals actually exists. In addition, inasmuch as the haploid males (1n) can be derived from the heterozygous females (2n) parthenogenetically, it is of interest to ascertain whether such males are uniform in their morphological characteristics. If dissimilarity does occur to any significant extent, dimorphism among the male population might then be attributed to differential action of the sex alleles (regions.)—Haploid males from a well inbred line *wh*+*gh* (sex alleles) were bred, and the number of microchaetae in the R_3 and 2d R_1 wing cell (Comstock) of the right mesothoracic wing were counted. Since bristles are unicellular secretions, the number of bristles within a specific area, 0.02275 sq. mm., is indicative of the number of cells present.—The resulting distribution of 600 animals was analyzed by the χ^2 test for goodness of fit to a normal distribution and gave $\chi^2 = 24.80$, $P > 5\% < 10\%$.—The failure of the distribution, the mean of which is 48.85 ± 0.152, to depart from normal, although close to being significantly different, vitiates the existence of a hidden bimodality, at least in this stock.—Apparently the sex alleles in this line do not exert a demonstrably different effect on cell size.

ROLLINS, REED C., Special Guayule Research Project, U. S. Dept. of Agriculture, Salinas, California: *Some known and probable levels of reciprocal introgression between guayule (Parthenium argentatum) and mariola (P. incanum).*——One of the principal sources of wide variation in guayulé under natural conditions is the interspecific crossing which occurs between it and mariola. The reverse is probably true to a lesser extent, but less is known concerning plants of hybrid origin among wild populations where mariola predominates.

Highly complicating factors to be dealt with in attempting to analyze the results of crosses between these species are the long polyploid series found in each species, and the crossability of plants from different chromosome levels. Chromosome numbers of $2n=36+$, $54\pm$, 58 ± 2, $72\pm$, and 108–$112\pm$ have been reported for guayule. In mariola chromosome numbers of $2n=36\pm$, $54\pm$, $72\pm$, $90\pm$, and $110\pm$ are known. Hybrids between these species, either natural or artificial, have been examined which have $2n=36\pm$, $54\pm$, $63\pm$, $72\pm$, $81\pm$, $90\pm$, $99\pm$ and $108\pm$ chromosomes. These hybrids have arisen from crosses between plants of guayule and mariola having the same or different chromosome numbers in various combinations. The expected chromosome number occurring in any given progeny is further complicated by the fact that both reduced and non-reduced egg-cells are functional in both species. Hybrids which receive a predominant number of maternal chromosomes resemble the mother plant instead of being intermediate between the two parents as is the case with hybrids receiving approximately equal numbers from each parent. Among the hybrids a very complex series is found which represents nearly a complete set of transitional steps from the one species to the other.

RUDNICK, D., Albertus Magnus College and Yale University, New Haven, Conn.: *The Creeper character in fowl as differentiated in limb grafts from early blastoderms.*——It is well known that matings of Creeper fowl ($Cp+\times Cp+$) yield three characteristic phenotypes: normal, Creeper (chondrodystrophic), and homozygous *Cp Cp*, which are fundamentally phokomelic although usually they die too early to display this character. In phokomelia, the whole body is affected, but the effect of the *Cp* gene is most strikingly manifest in the limbs. In the normal chick blastoderm during early somite stages, areas capable of forming wing or leg respectively are localised in the future trunk region. This has been shown by transplanting measured pieces to the embyronic coelom of a 54–60 hour host, and growing them from 8–10 days. Such graft limbs are apt to be distorted and fragmentary, but individual cartilages may differentiate in characteristic gross form, and in particular the number and distinctness of phalanges is strikingly normal. When the leg-forming region of young blastoderms (5–18 somites) from a $Cp+\times Cp+$ mating is similarly isolated and transplanted to the coelom of a non-Creeper host, close to 25 percent of identifiable legs are typically phokomelic, with proximal cartilages much reduced and the digits occupied by solid cartilages instead of discrete phalanges. The 75 percent showing normal digits cannot always be certainly classified as normal or Creeper. Wing grafts show a similar although less clear-cut segregation into phokomelic and non-phokomelic types. Thus the major aspect of the homozygous Creeper phenotype is localized very early in the limb region and is clearly independent of the Creeper blood circulation.

SANSOME, E. R., A. HOLLAENDER, E. ZIMMER, and M. DEMEREC, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Industrial Hygiene Research Laboratory, National Institute of Health, Bethesda, Md.: *Quantitative effects of radiation on mutation production*

in Neurospora crassa.——The heritable nature of alterations produced in fungi by monochromatic ultraviolet irradiation was verified by genetic analysis of a fungus having a sexual stage. Microspores of the "fluffy" type of *Neurospora crassa* were used in this study. The effects of wave length and dosage on the mutation rate resembled those reported for some Fungi Imperfecti. To eliminate contaminants, the mutants were routinely crossed with the conidial "wild" type. Certain of the ascospore progeny were germinated and studied in more detail. Of the 40 crosses, where the ascospores were sown at random, more than one type of "fluffy-mutant" was recovered from about half. This suggests a high coincidence of mutations. Unstable mutants were subjected to microspore analysis and classified.—Experiments with X-rays, indicate that visible mutants were produced with a frequency which increased linearly with dosage. For dosages given at an intensity of 270 r per minute, the frequency of mutants increased from 2.8 per cent at 2,250 r to 24.6 per cent at 31,500 r. About half the spores survived the treatment of 31,500 r. When dosages up to 126,000 r were given at 5,400 r per minute, the mutation rate increased to 78.5 per cent with a survival of 0.01 per cent. The observation of the LINDEGRENS that X-ray-induced mutants, when crossed with the wild type, often gave more aborted ascospores than the control crosses was confirmed. It was found that treated cultures which were phenotypically normal were also sometimes sterile, but that the proportion of steriles among the mutants was much higher than among the normals.

SAWIN, PAUL B., Brown University, Providence, R. I.: *Regional amplitude and specificity of influence of genetic factors inducing homoeotic variations in the axial skeleton of the rabbit.*——Variations in the number of ribs and presacral vertebrae have been compared in three races of rabbits in their hybrid and several subsequent generations. Neither variation in itself can be considered to be transmitted in a simple Mendelian manner. When the two variations are considered together from the broader viewpoint of heteromorphic (homoeotic) tendencies evidence is found in one family of a more general influence producing extra skeletal units at both the thoraco-lumbar and lumbo-sacral borders as has been described in man by KÜHNE. In the other two families the genetic differences appear to be more restricted in range, in the one to the thoraco-lumbar border, in the other to the lumbo-sacral. The observation of a specifically different heteroauxetic growth range in the more central regions of the body in each of these families seems to indicate that the most probable mode of gene action in causing these variations is by means of localized embryonic regions of growth and that the number of factors may be large as in the case of general body size.

SCHULTZ, JACK and FAYE STEWART JOSE, The Lankenau Hospital Research Institute, Philadelphia, Pa.: *Differentiation of chromosomal proteins by staining techniques.*——The giant chromosomes of the salivary glands of the Diptera contain differentiated structures usually distinguished by their nucleic acid content. The nucleoprotein bands of various types are separated by the protein interband regions, giving a characteristic pattern along the chromo-

some. A group of acidic dyes—fast green, acid fuchsin, aniline blue, chlorazol black E, and others—has been used in staining the salivary gland chromosomes of various species of Drosophila. These dyes do not stain nucleic acid under the conditions of the experiments, yet the same patterns were found in the chromosomes as were already familiar from the nucleic acid tests. They do stain proteins, however. It follows that the nucleoproteins may be distinguished from the interband proteins by the staining reactions of their protein moieties; the nucleoproteins stain heavily with acid fuchsin or fast green, the interband proteins lightly with acid fuchsin and not visibly with fast green. There is in addition to these another protein, non-fibrous, which has been called the "matrix" protein. It stains with the acidic dyes; its distribution in the chromosomes parallels that of the component whose ultraviolet absorption spectrum is characterized (CASPERSSON) by a shift of the protein band towards longer wave lengths. There are complexes of bands in the salivary gland chromosomes called capsules, in which morphologically identical bands are associated around their edges—a protein space within, as filler to the neucloprotein pie crust. Since the matrical protein stains with fast green and the fibrous interband protein does not, the consistent absence of the stain within the capsules indicates a different distribution of protein within and between capsules. Tests show that the "matrical" protein is easily digested off the chromsomes by pepsin and ribonuclease, and is readily soluble in buffers at pH 2. On immersion in buffers of higher pH than 7 acetic acid fixed and smeared chromosomes show a progressive disintegration of structure. It is shown that the interband proteins are involved in this, indicating that the fibrous proteins of the interbands dissolve at pH's where histones precipitate.

SCOTT, J. P., Wabash College, Crawfordsville, Ind.: *Experimental modification of aggressive and defensive behavior in the C-57 inbred strain of mice.* (Kodachrome moving picture.)——During the course of experiments whose outcome supported the theory that social organization is the result of social behavior, the following results were obtained from attempts to control defensive and aggressive behavior in males from subline 10 of the C-57 strain. (1) Relationship between cage space and pattern of defensive behavior: an inexperienced male familiar with a large multiple-escape pen attempts to escape by running when attacked and beaten by an experienced aggressor. The same mouse confined to a small pen assumes an upright defensive posture (the so-called "submission reaction") when attacked and runs or jumps a short distance away if hurt. The defensive posture appears to be adaptive in nature, discouraging attack and holding off the aggressor. (2) Relationship between training and aggressive behavior: a male raised with a female since weaning and handled for 3–5 days preceding the introduction of a strange male does not start a fight. The same mouse isolated for two weeks in another pen and subjected for five days to light attacks by an aggressive mouse will fight the male with which it was previously peaceful. Certain other training procedures appear to increase the strength of aggression. This is a case in which the hereditary background was constant and aggressive and defensive behavior were extensively modified by environmental means.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Mich.: *Inheritance of spotless pattern in Hippodamia convergens, and its frequency in several populations.*——The spotless pattern in its simplest form differs from spotted by only one gene, with spotless almost completely dominant. There may, however, be modifying genes which restore to spotless some, or even all, of the spots in reduced size. The modifiers appear to have no effect on the spotted pattern. The two patterns are most sharply distinguished by the three posterior spots, but there may be overlapping even of these if modifiers are present with the spotless. For statistical purposes in this study spotless could be separated from spotted in the worst instances of overlapping at the point where the posterior spots have about two-thirds of their maximum size. About 6 or 7 percent of the pertinent chromosomes contain the spotless gene in Michigan and, by the Michigan standards, also in Colorado; but only 2 to 3.5 percent contain it in California. The modifying genes in Colorado may be different, so as to modify the above judgment concerning that region. There appear to be as many as four pairs of modifying genes in the Michigan beetles in some of the experiments, fewer in others, and none at all in some. The modifying genes do not all have the same effect; one of them affects one spot in particular, others affect other spots. On the basis of certain assumptions it is computed that the modifiers are nearly three times as frequent in Michigan as in California.

SNYDER, LAURENCE H. and C. A. DOAN, Ohio State University, Columbus, Ohio: *The apparent lethal nature of the gene for telangiectasia in man.*——A case is presented of severe generalized multiple telangiectasia in a newly born female infant. The condition was incompatible with life, death occurring at eleven weeks. Classical multiple telangiectasia of the usual mild type occurred in the paternal grandmother, the maternal grandfather, and both parents. The family history suggests the probability that this case represents the first recorded instance of the homozygous form of the dyscrasia, and that the gene for multiple telangiectasia is lethal when homozygous.

SONNEBORN, T. M. Indiana University, Bloomington, Indiana: *Evidence for a bipartite structure of the gene.*——The following evidence indicates that the cytoplasmic factor, kappa, required in addition to gene *K* to determine the "killer" character in variety 4 of *Paramecium aurelia*, occurs in the macronucleus as well as in the cytoplasm. When a small amount of kappa is introduced into a non-killer cell having the killer gene (*K*), the resulting clone retains and reproduces kappa, but remains non-killer. Failure to become killer is due, not to insufficient kappa in the cells, but to insufficient (or no) kappa in the cytoplasm; for the amount present in the cells is far more than enough to determine as killers, if all of it were present in the cytoplasm. Hence, some kappa must be present in the macronucleus. (Previous work shows it is not in the micronucleus.) The following evidences indicate not only that kappa is in the macronucleus, but that in the macronucleus it is bound to gene *K*: (1) kappa is in the macronucleus only when the macronucleus contains K; (2) in the macronucleus, kappa is reproduced at the same rate as *K* (kappa

doubles at every gene doubling); (3) by varying the concentration of kappa and the number of *K* genes per cell, the absorption of kappa by *K* is demonstrable. Comparison with conditions in variety 1 and in higher organisms leads to the view that genes normally consist of two parts corresponding in their functions to *K* and kappa.

SPARROW, A. H., Harvard University, Cambridge, Mass.: *Reduced chromonema elongation and abnormal spiralization following X-ray treatment of meiotic chromosomes.*——In Trillium, the formation of the major coil between early diakinesis and first anaphase is normally accompanied by an elongation of the chromonema estimated to be not less than threefold. However, in pollen mother cells X-rayed at meiotic prophase the major spiral in many cells is very poorly developed even as late as second anaphase. Measurements of chromonema length at first and second anaphases in such cases indicate that elongation has been reduced to considerably less than half that found in control material. Reduced spiralization in other cases is known to be associated with low nucleic acid concentrations, e.g., in negatively heterochromatic chromosomes or regions and in cold-induced "differential reactivity." It is also known that X-rays inhibit the formation of desoxyribose nucleic acid. It is therefore suggested (1) that the abnormal spiralization and reduced chromonema elongation following X-ray treatment may be associated with an X-ray induced deficiency of desoxyribose nucleic acid, and (2) that the normal prophase increase in desoxyribose nucleic acid may play an important role in the mechanics of spiralization.

STADLER, L. J., and SEYMOUR FOGEL, U. S. Department of Agriculture and University of Missouri, Columbia, Missouri: *Gene variability in maize.* II. *The action of certain R. alleles.*——Alleles of *R* extracted from unrelated stocks differ widely in their effect upon distribution and intensity of anthocyanin pigmentation of the plant. This variation is independent of the aleurone color effect, a similar range for plant color being found among R^r's (colored aleurone) and r^r's (colorless aleurone). Among R^r's distinguishable levels of aleurone color effect occur.—Plant pigmentation determined by a given allele may be greatly altered by environmental factors and modifying genes. By comparison of sib populations in cultures of the type $R^r/r^r \times r^g$ these difficulties are minimized, and minute differences in allelic action may be recognized and objectively tested.—Twenty-two R^r alleles (these Records, 11: 90, 91), constituting a virtually unselected sample of R^r's of independent origin, were found to include few if any duplicates in phenotypic action.—The alleles form a substantially linear series in their effect upon the tissues chiefly involved in the coloration of seedling organs and of mature leaf sheath, blade margin, husk, glume, and anther. Certain alleles produce only faint pigmentation, limited to a few tissues; others produce pigmentation of varying intensity in a wide variety of tissues. The effects of the entire series of alleles on these tissues may be fairly well represented in a single graph in which an arbitrary potency value is assigned to each allele and a hypothetical threshold requirement to each tis-

sue.—The plant color effect of the same allele is decidedly non-linear in certain respects. One allele, intermediate in the coloration of the tissues referred to above, produces intense pigmentation of pericarp, silk, and leaf collar. Two others among the 22 R^r's produce full (non-variegated) pericarp color (both at a lower level of intensity), and in these also the color of silk and leaf collar is more intense than would be expected from their position in the series for other tissues. These alleles were studied in combination with several r^r's selected for their pericarp effect. Within this group also a linear relation appears, affecting the pigmentation of various tissues in leaf sheath, culm and glume, in addition to those in pericarp, silk, and leaf collar.—The action of the *R* alleles may thus be viewed as including three more or less independent components, one identified by the effect upon aleurone color, another chiefly by seedling coloration, and a third chiefly by pericarp color. Each component may occur at any one of various levels, and apparently any combination of levels of the three components may occur together in a single allele. There are indications of the existence of additional components. Although the different alleles behave as if composed of three or more completely linked genes or subgenes, it is not implied that these components represent independent units rather than different aspects of the action of a single unit.

STALKER, H. D., Washington University, St. Louis, Mo.: *Genetic studies on Scaptomyza graminum.*——*S. graminum* is a leaf-miner which belongs to the family Drosophilidae, and feeds on deciduous plants. The metaphase chromosomes as described by METZ, are similar to those of *D. melanogaster*, except that in some Rochester, N. Y. strains, the microchromosomes were short rods, rather than dots. These rod-shaped microchromosomes apparently had no deleterious effect on the carriers, although all combinations of rods and dots were found. Wild females, fertilized in nature, were collected from the Rochester and St. Louis, Mo. areas; fifty-two visible mutations were found in the F_2 or as spontaneous occurrences in the laboratory. Of these, two were autosomal semi-dominants, three sex-linked recessives, and 47 autosomal recessives. The average number of visibles per wild female was .88. Only 31 of the mutations showed complete penetrance, *S. graminum* resembling *Drosophila funebris* in this respect. One sex-linked and two autosomal linkage groups were found; as in Drosophila, there was little or no crossing over in the male. Four visibles found in the Missouri females showed frequencies of 38, 8, 6 and 6 percent respectively. Their distribution was apparently continuous and suggested a high degree of homogeneity among the Missouri sub-populations. Such homogeneity was not apparent in the two New York sub-populations. The difference may be due to the more drastic effect of droughts in Missouri which eliminate the flies over a larger area, so that when the population is rebuilt, many sub-populations arise from a single surviving nucleus.

STECHER, ROBERT M., and A. H. HERSH: *The inheritance of human gout or the incidence of familial hyperuricemia.*——Although gout produces arthritis, it is fundamentally a metabolic disease due to abnormal uric acid

metabolism resulting in a high blood level. The disease is hereditary but a clear mechanism of inheritance is obscured by imperfect penetrance. We have surmounted the difficulty by recognizing a hereditary hyperuricemia making determinations on serum, instead of waiting for clinical arthritis.

The incidence of hyperuricemia was determined in parents, siblings and offspring of gouty people. Of 30 families studied, two of four fathers and three of seven mothers were said to have had gout or had hyperuricemia. Six of 17 brothers, seven of 18 sisters, six of 20 sons but none of 40 daughters were affected. These data are being extended constantly.

The incidence of hyperuricemia in the general population was determined by routine observations of patients as they entered the medical service. After corrections, necessary because hyperuricemia without gout may occur in leukemia, acute hemorrhage and kidney insufficiency, we found only 15 or 3.7 percent of 407 white people examined had hyperuricemia. Hyperuricemia occurs with nearly ten times the normal frequency in brothers, sisters and sons but not in daughters of gouty patients. We infer that some factor besides hyperuricemia is necessary to produce clinical gout. It is hoped that the mechanism of inheritance will become apparent as these investigations are extended to include studies of pedigrees, and gene frequency analyses.

STILES, KARL A., and JEAN SCHALCK, Coe College, Cedar Rapids, Iowa: *A pedigree of curbed forefingers.*——Curved forefingers are characterized by a variable deviation to the ulnar side of the two distal phalanges of one or both index fingers. This trait has been traced through four generations. In this pedigree the distribution and ratio of individuals with curved forefingers is in complete harmony with a single gene interpretation, in which this particular type of character is assumed to be completely dominant to the straight fingers. There is considerable variation in the amount of curvature of the forefingers among the individuals showing the character. The cause or causes of variability of the phenotypes produced by what appears to be a single gene is an open question. However, the variability of the curved fingers in this family is not so great but that it might be due entirely to environmental factors.

STORMONT, CLYDE, M. R. IRWIN, and RAY D. OWEN, University of Wisconsin, Madison, Wisconsin: *A probable allelic series of genes affecting cellular antigens in cattle.*——Approximately 40 antigens are now recognized in the red blood cells of cattle. The majority of these are detected by their reaction with specific reagents, each of these being obtained by antibody absorption of immune serums produced either in cattle or rabbits. Although each antigen appears to be an immunological entity, certain of them are not genetically independent. For example, the antigenic characters called *B*, *G* and *K* have appeared to date only in the following combinations: *B* alone, *G* alone, *BG* together and *BGK*, as well as the absence of all three. Thus *K* has been found only in the simultaneous presence of both *B* and *G* (only one questionable exception in 633 individuals containing *BGK* out of 2051 cattle tested). Furthermore, genetically there appear to be two kinds of individuals carrying

both *B* and *G*. These individuals are most easily differentiated if heterozygotes, by virtue of the types of offspring produced in matings to animals not containing either character. They are, respectively, (a) those whose offspring also possess either the complex *BG*, or neither, or (b) those whose offspring possess only either *B* or *G*. These results suggest that a single gene may produce the two antigens *BG* in some animals, and these are immunologically indistinguishable from individuals which carry separately the probable alleles, *B* and *G*. (The progeny from critical matings of these two presumptive kinds of *BG* individuals are relatively few.) It is proposed that these antigens (*B*, *G* and *K*) are produced by an allelic series of genes; one for the absence of all three antigens, one for antigen *B*, one for *G*, one for *B* and *G* together (that is—a single gene having the same effect as a pair of alleles with contrasting effects), and one for the *BGK* complex (a single gene producing three antigenic entities). The possibility of linkage in this case cannot be excluded, but at present seems less likely. Ten or more other antigens appear to belong in this series.

STRONG, L. C., Yale University Medical School, New Haven, Conn.: *The influence of methylcholanthrene on the genetic constitutional state of susceptibility to cancer of the stomach in mice.*——Two hundred thirty-five cases of cancer of the stomach have been obtained in mice of the NHO strain following the subcutaneous injection of 1 mg of methylcholanthrene dissolved in sesame oil. No similar lesion has been encountered in the original control series of the same strain. However, the descendents of those mice developing cancer of the stomach in the methylcholanthrene treated series now develop cancer of the stomach, not only after the administration of methylcholanthrene subcutaneously but also spontaneously. Two genetic explanations seem reasonable; (1) that the methycholanthrene has been used as a tool to detect minute genetic potentialities from the germ plasm which by selection from hybrid mice have now become so pronounced that their somatic manifestation, cancer of the stomach, appears spontaneously, (2) that methylcholanthrene has actually produced or brought about a genetic change or changes in the germ plasm which is inherited. The somatic appearance of this genetic change is the same as that brought about by induction with the original methylcholanthrene treated mice. The relationship of the present data to the old problem of "the inheritance of acquired characters" is obvious and the final interpretation should be of interest not only in oncology but also in genetics.

VILLEE, CLAUDE A., University of North Carolina, Chapel Hill, N. C.: *Interactions of homoeotic mutants with certain "growth rate" genes of Drosophila melanogaster.*——The development of the homoeotic mutants aristapedia (ss^a), aristapedia-Bridges (ss^{aB}), proboscipedia (*pb*), and bithorax (*bx*) may be altered by exposing the larvae to high or low temperatures and thus increasing or decreasing the growth rate (VILLEE). WADDINGTON described four genes affecting growth rate: dachs (*d*), dachsous (*ds*), fourjointed (*fj*), and combgap (*cg*). All of these have some effect in shortening or distorting the legs and in addition have effects on body and wing size and shape. Stocks combining these

growth rate genes with the several homoeotic mutants were set up. Two types of interaction were found: certain of the homoeotic mutants increased the effect the growth rate genes normally have in shortening the legs, and certain of the growth rate genes increased or decreased the effect the homoeotic genes normally have on their respective structures. Both ss^{a} and ss^{aB} increased the reduction of the tarsus normally produced by *d* and *fj* so the tarsi were very short, consisting of one or two segments only. A lesser interaction was observed between ss^{a} or ss^{aB} and *ds* or *cg*: the tarsi of such combinations varied from 3–5 segments. No increased shortening of the legs was observed in combinations of the growth rate genes with *pb*. The stock *ds* ss^{aB} shows a marked increase in the expression of aristapedia over ss^{aB} alone. Smaller increases were caused by *d* or *fj* in combination with ss^{aB} and the antennae of *cg* ss^{aB} were identical with ss^{aB} alone. The expression of ss^{a} was reduced by *fj*, *d*, and *cg*. A change in growth rate induced by these genes has the same effect on a homoeotic mutant as one induced by a change in temperature during development.

WARMKE, H. E., Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Studies on assay and inheritance of the marihuana drug.*——In cooperation with the Department of Agriculture, an attempt is being made to breed a strain of fiber hemp, *Cannabis sativa*, with greatly reduced drug content. After three generations of continued selection and crossing of low- and of high-content plants, strains have been developed which differ from each other in average marihuana potency by a factor of four. The low-content strains are significantly less toxic than the parent stocks from which they were derived. Drug content, therefor, appears to be heritable, and it does not seem to be related to fiber quality. The toxicity of individual plants is measured by a method of bioassay in which the water flea, *Daphnia longispina*, is used to test minimum lethal concentrations of standard leaf extracts.

WHITTINGHILL, M., and ERNESTINE REUTTER PARKER, University of North Carolina, Chapel Hill, N. C., and Laboratories of Bacteriology, New York Post-Graduate Medical School, New York, N. Y.: *Effects of selection and of temperature upon a new eye-shape mutant in Drosophila melanogaster.*——An eye-shape mutant of variable expression has been found to be due to a gene located near lobe. It appeared as a single male having kidney shaped eyes in a *ClB* stock. Cultures are maintained by selection. Expression may vary from the normal oval to eyes which are double kidney, or split all the way through the middle, or reduced to an upper lobe only. Such wide variation is rarely found in a single stock. Right and left eyes often have different shapes. The flies have been classified according to the total number of indentations in the two eyes as flies of grade 0, 1, 2, 3, 4, and extreme 4. The stock averaged grade 2 at the start of the selection experiments. Selection for increased numbers of indentations resulted in shifts during a period of five generatons until the flies were mainly of grade 4. Temperature experiments with this stock revealed that the mutant had a greater effect at 28°C than at 25°. Selection in the opposite direction produced several stocks which, in the fifth and eighth generations,

consisted mainly of normal-eyed offspring with a few of grade 1. Tests with the selected stock showed no allelism with *std*, *sca*, nor with *vg*. An earlier, negative test to lobe will be superceded by one in which crossovers will be distinguished from normal overlaps. Until such allelism may be ascertained, the new mutant is known by the name "indented."

ZAMENHOF, S., 620 W. 115th St., New York, N. Y.: "*Mutations*" *and cell divisions in bacteria.*——This study deals with the problem whether n bacteria "mutations" can occur without cell divisions. The writer worked on his new strain of "unstable variant" and on several other strains of *B. coli mutabile* (Parr); the method used was devised to eliminate other phenomena such as multiplication of "mutants," natural selection, period of time necessary to produce medium preferential for the "mutants," etc.—Following facts were established: (1) The occurrence of "mutations" ceases if cell divisions are suppressed by means of lower or higher temperature or foodless medium; upon restoration of cell divisibility the "mutability" resumes regardless of the length of period of non-division. This suggests that no visible or latent "mutation" occurs during the non-division period. (2) The number of "mutations" increases with the number of cells present, number of hours and temperature, but only as long as these factors increase the number of cell divisions during the "mutability" period; it seems therefore proper to express the "mutability" of bacteria as number of "mutants" per number of parent cell *divisions* in this period, rather than per number of parent cells and per time unit. (3) In strains other than "unstable variants" the"mutability" starts long after the logarithmic period (Parr), even if the medium is always preferential for the "mutant." The reason may be inherent in the old age of cells; in such strains the old cells might divide "improperly" forming "mutants." This would support the theory of REED that at least some "mutations" of bacteria are "aberrations of genic material at division (or duplication)" rather than "point mutation."

THE EMBRYONIC DEVELOPMENT OF MUTANTS OF THE *Sd*-STRAIN IN MICE[1]

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A SYNDROME of abnormalities in the mouse has been described (GLUECKSOHN-SCHOENHEIMER 1943) in which defects in the spinal column, the urogenital system, and the intestine are associated together in animals which are heterozygous or homozygous for a single mutation, *Sd* (DUNN, GLUECKSOHN-SCHOENHEIMER, and BRYSON 1940). These defects—as apparent in the mutant mice between birth and adult life—consist in reduction or absence of the tail, spina bifida, and vertebral abnormalities, while reduction or absence of kidneys, presence of a cloaca, absence of rectum and imperforate anus are the abnormalities found in the urogenital system and the intestine. The homozygotes, which are always grossly defective, die shortly after birth, while heterozygotes may suffer a high mortality in early life.

The abnormalities as found in these newborn and adult mice represent the end result of a chain of events at the beginning of which stands the gene. The analysis of the action of the gene is our ultimate goal; at the present, however, we must content ourselves with the description of as many visible effects as possible of the chain of processes started by the gene. Beginning with the end result—that is, the malformations in the newborn—we trace back the developmental history of the abnormal organs throughout embryogeny until we come to a stage where morphological methods are not sufficient to distinguish the abnormal from the normal. A further analysis of the processes bringing us closer to the gene must be left to experimental rather than descriptive methods.

The present paper deals with the embryonic development of the abnormal organ systems in heterozygous and homozygous *Sd* mice. The organs affected are the spine, the urogenital system, and the intestine. We know of several other mutations producing malformations in the sacral and caudal parts of the spine; the processes which in the different mutants lead to the same end results (for example, tail-shortening, fusion and abnormalities of individual vertebrae) will be compared with each other.

The author wishes to thank Dr. L. C. DUNN for his stimulating interest and help during the course of this work.

MATERIAL AND METHODS

The material for the embryological investigations was obtained from matings of heterozygous animals (*Sd* +) *inter se* and from outcrosses of heterozygotes to normals. The embryos were timed and obtained by the methods described previously (GLUECKSOHN-SCHOENHEIMER 1938).

[1] This research was aided by grants from the JOSIAH MACY, JR., Foundation and the Fund for Research of COLUMBIA UNIVERSITY.

Embryos were studied between the ages of eight days and 17 days after fertilization. Sixty-two litters were dissected, and a total of 576 embryos was obtained. Of these, heterozygous (*Sd* +) embryos up to and including the 11th day after fertilization could not be diagnosed with absolute certainty as to their genotype in regard to *Sd*. The distribution of 241 embryos of 12 days and older among the different classes obtained from crosses of *Sd* + to normal and to *Sd* + was as follows: (1) from crosses of *Sd* + by ++:88 *Sd*+ 86++; (2) from crosses of *Sd* + by *Sd* +:20 ++ 29 *Sd* + 18 *Sd Sd*, the departure from the expected ratio of 1:2:1 not being significant.

All embryos were fixed in Bouin, imbedded in paraffine, sectioned at 8μ, and stained in Delafield's haematoxylin and eosin.

DEVELOPMENT OF THE HETEROZYGOUS SHORT-TAILED OR TAILLESS EMBRYOS (*Sd* +)

Tail

The development of the prospective short-tailed or tailless heterozygous embryos progresses normally up to the tenth day. At the age of about 10½–11 days differences between normals and heterozygotes become apparent externally, the heterozygotes showing slight abnormalities. The last few somites of the tail may appear small and irregular, and small haematomata are found in the posterior part of the tail. The tail somites degenerate progressively, starting distally and proceeding in proximal direction. A gradual decrease in the diameter of the tail may be observed, the more distal portions shrinking rapidly and the shrinking progressing proximally. Extravasation of blood in distal portions of the tail appears in the early degenerative stages (ten days); the haemorrhages increase in size and may sometimes fill one third to one half of the entire tail (fig. 1). No sharp point of constriction between normal and abnormal part of the tail, as described for the Brachyury mutation (CHESLEY 1935), can be found in the *Sd* + embryos, but the tail becomes thinner gradually. The distal abnormal portion of the tail may persist as a filament throughout the life of the abnormal mouse.

Histologically, abnormalities in the tail of the heterozygotes may be observed slightly before they become apparent externally. The distal somites of the tail at the age of ten days contain pycnotic granules; these granules can also be found in the mesenchyme between and around the somites and in the neural tube. Simultaneously, the small haemorrhages containing blood extruded from the vessels appear in the distal part of the tail. But cell pycnosis was observed also in the absence of haematomata. The number of pycnotic granules and the size of the blood extravasations increase as development proceeds and the distal somites appear now small and irregular. Eventually, all structures in the affected part of the tail break down, such as neural tube, notochord, tail gut and blood vessels (fig. 3, 4). The degeneration of all these structures sets in simultaneously at the age of 11 days and is a secondary process following the process of cell necrosis in the neural tube, somites and mesenchyme, and the appearance of extravasated blood.

Notochord and intervertebral discs

The entire notochord of the embryo degenerates subsequent to the appearance of abnormal processes in the tail. In sections of embryos of the 11th day the notochord appears discontinuous; it breaks up and eventually disappears throughout the length of the animal, leaving behind only scattered remnants consisting of two or three abnormal cells each. As a result of the disintegration of the notochord, a normal nucleus pulposus fails to appear. The significance of the absence of the notochordal remnant during the development of the intervertebral disc remains unknown. Apparently, the development of the intervertebral disc is not greatly disturbed by the absence of the nucleus pulposus, because no abnormalities other than an occasional lordosis, kyphosis, or scoliosis or occasional fusions of individual vertebrae were observed in the spine of adults. Since the notochord and thus the nucleus pulposus is abnormal at all levels of the spine, the normal development and function of any intervertebral disc would indicate that the nucleus pulposus is not an essential part of the intervertebral disc.

Sacral region

No gross abnormalities were observed in the sacral region of the heterozygous embryos; the development of the vertebrae from the somites was not studied in detail.

Urinary organs

In view of the variety of abnormalities of the urogenital system in the adult (GLUECKSOHN-SCHOENHEIMER 1943), corresponding variations of developmental abnormalities in the embryo are to be expected. The development of the urogenital system of *Sd* heterozygotes is normal up to the time when the ureters bud off from the mesonephric ducts. The first abnormalities were found in embryos at the age of ten days; one embryo which on the basis of tail abnormalities had been classified as *Sd* + had a short, round and broad ureteric bud instead of the narrow long bud with the widened distal end (that is, the primitive renal pelvis) found normally. Another embryo from the same litter showed duplication of ureteric buds, the secondary bud coming off from the mesonephric duct slightly posterior to the primary bud. This duplication of ureteric buds is a frequent occurrence in the heterozygotes (fig. 7). The secondary bud is always shorter than the primary one; it does not connect with the metanephros and does not become functional; this finding agrees with the fact that double ureters were never found in adult heterozygotes.

At the age of 11 days after fertilization the widened distal end of the normal ureter—that is, the primitive renal pelvis—begins to branch and the cranial and caudal pole tubules are formed (fig. 6). The metanephrogenic tissue which is located around the ureter tip forms little caps around the tips of both the cranial and the caudal pole tubule. The caudal and cranial pole tubules divide again, each branch carrying the cap of metanephrogenic tissue around its tip. The pole tubules develop into the collecting tubules—that is, the excretory system of the kidney—and the caps of metanephrogenic tissue form the secretory elements—namely, the secretory tubules and renal corpuscles.

In the heterozygous mutants (*Sd* +) the development of this system of tubules is inhibited entirely or disturbed. In the case of complete inhibition, the distal end of the ureter (the primitive renal pelvis) does not branch at all and no tubules develop (fig. 7). In other cases, the inhibition is not complete and the branching of the ureter tip proceeds to a certain extent, more slowly than in the normal and resulting in a smaller number of tubules. Once the process of branching is initiated, each tubule develops normally into an excretory element, with the metanephrogenic tissue covering its tip and finally differentiating into the secretory tubules and renal corpuscles. Of the two systems of the metanephros, the secretory and excretory, the secretory part of the metanephros never develops in the absence of the excretory part, in spite of the presence of the metanephrogenic tissue in all heterozygotes. Its formative development does not take place unless it is preceded by the formation of the excretory system from the distal end of the ureter. If the ureter is missing completely or if its distal end does not branch at all, absence of the kidney will result from it; in case branching does take place, the size of the kidney will depend on the extent of the branching process and the number of tubules resulting from it.

DEVELOPMENT OF THE HOMOZYGOUS EMBRYOS (*Sd Sd*)

Tail

The homozygous *Sd Sd* embryo can be distinguished from its normal and heterozygous litter mates at the end of nine days after fertilization by macroscopic examination. At that stage, the tail bud begins to elongate in both the normal and the homozygote, but in the homozygote a constriction appears at the tail base. The constricted tail is shorter and thinner than the normal tail. It is characterized by haematomata of different sizes in its distal part. These haematomata can be seen on macroscopic examination. In sections of the tail bud at nine days all structures of the tail are clearly abnormal: the notochord does not appear as a continuous rod, but only traces of it are found, while in earlier stages it appears perfectly normal; the neural tube extends into the tail as does the tail gut, and somites are present. All these tail structures contain large numbers of pycnotic granules. These can be found in the neural tube, around the somites, inside the somites, and all through the tail mesenchyme (fig. 5). The number of pycnotic granules increases as the degenerative processes in the tail continue; the haematomata become bigger due to the addition of more blood extruded from the blood vessels; eventually all the structures break down, and at the end of 12 days only a filament is left with one or several large haematomata inside which are gradually resorbed. Due to the haematomata the tail appears swollen in spots and shows alternating bulges and constrictions (fig. 2).

Sacral region

The sacral region of the *Sd* homozygotes is characterized by the failure of the neural folds to close; a cleft is visible between the neural folds covered by a large transparent bleb of the epidermis. This embryonic abnormality is directly connected with one of the malformations regularly encountered in the

newborn *Sd* homozygotes—that is, spina bifida (GLUECKSOHN-SCHOENHEIMER 1943). Another characteristic external feature of the *Sd* homozygotes at the age of 11 and 12 days after fertilization is the failure of development of the genital papilla which in the normal embryo first appears at this age and grows during the succeeding days. Thus, by the age of 11 days the homozygous *Sd* embryo is easily recognized externally by the abnormal appearance of the tail, by the absence of the genital papilla, and by the large bleb above the cleft neural folds in the sacral region.

Urogenital System of the Homozygotes

The abnormalities in the developing urogenital system of the homozygotes are more extensive than those described for the heterozygotes just as the urogenital malformations in the homozygous newborn are more extensive than those of the heterozygotes. At the end of nine days or the beginning of ten days after fertilization the mesonephric ducts are frequently abnormal in their posterior portion; they do not reach beyond the point where the ureter bud comes off and do not establish a connection with the cloaca. This posterior part of the mesonephric duct is normally taken up into the bladder, and the abnormalities of the bladder described for the homozygous newborns (complete absence or abnormally small size) may well be connected partly with the failure of the posterior part of the mesonephric duct to develop. The completely normal appearance of the gonads and their ducts, on the other hand, shows that the mesonephros and the anterior part of the mesonephric duct which are normal morphologically are normal in their developmental potencies as well.

The ureters bud off from the mesonephric duct normally; but in most cases two buds come off from each mesonephric duct. This duplication of ureteric buds is more frequent than in the heterozygotes and is almost a regular feature of the homozygotes. Both primary and secondary ureters are underdeveloped, one usually more than the other; both stay short, turn dorsad and caudad, and usually the tips do not branch at all; occasionally cranial and caudal pole tubules develop, and even more rarely a few secondary tubules appear (fig. 8). Nephrogenic tissue is found around the tip of the ureter, but it fails to undergo any further differentiation, except for the rare cases in which some differentiation of the ureteric tip (=renal pelvis) occurs. The rather infrequent appearance of a very small solitary kidney in the newborn homozygotes reported before is linked up with the occasional slight differentiation of the ureteric bud.

The cloaca is abnormally small in the *Sd* homozygotes of about ten days. It does not grow normally, and its separation into urogenital sinus and rectum fails to take place. Occasionally, the vesical portion of the urogenital sinus develops to a certain extent, and thus can be explained the few cases of newborns which do contain a bladder, however small. The absence of the bladder in most cases and the failure of the rectum to develop thus go back to abnormalities of the cloaca in embryos of 11 and 12 days. The absence of genital papilla and anal opening are probably direct results of the absence of bladder and urethra and of the rectum.

DISCUSSION

In the development of the *Sd* mutants, both heterozygotes and homozygotes, two organ systems are affected, the axial skeleton and the urogenital system. As to the axial skeleton, it seems from the purely morphological evidence that the abnormal process affecting the tail in embryos of the *Sd* mutants differs from the abnormal processes which have been described for other tail mutants. The embryonic development of tail mutants has been investigated for the following mutations: flexed (*fl*), Brachyury short tail (*T*), and the two recessives t^0 and t^1. KAMENOFF (1935) showed that the presence of the flexes or fusions between contiguous vertebrae in the tails of adult flexed mice can be traced back to abnormalities in the intervertebral discs of flexed embryos: part of the cartilage of the intervertebral discs fails to differentiate into fibers. Although abnormal bending and branching of the notochord was observed in some of the flexed embryos, these abnormalities have no causal relation to the fusions of the vertebrae. No degenerative changes were observed in the tail region of flexed embryos.

A study of the development of mice carrying *T* alone (short-tailed mice) or *T* and t^0 or t^1 (tailless mice) showed that the notochord is the organ primarily affected in these mutants (CHESLEY 1935; GLUECKSOHN-SCHOENHEIMER 1938). In embryos of these mice the tail is at first formed normally from the tail bud; at the 11th day of development, however, a constriction sets in somewhere along the tail in the short-tailed mice ($T/+$) and at the tail base in the tailless mice (T/t^0 or T/t^1) which results in a resorption of the tail distal to the constriction. The absence of the tail and the abnormalities of the neural tube, somites, and other structures in the constricted part of the tail are secondary and can be traced back to the original abnormalities of the notochord.

The morphological picture produced in the posterior trunk and tail region of the *Sd* mutants indicates the presence of abnormal processes different from those in flexed, Brachury, and tailless mice. In *Sd* a cellular degeneration proc-

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EXPLANATION OF FIGURES

FIG. 1.—*Sd*/+ embryo. 12 days. Note haematoma in distal part of abnormal tail. ×9.

FIG. 2.—*Sd*/*Sd* embryo. same age. Note abnormal tail and absence of genital papilla. ×11

FIG. 3.—Sagittal section of tail of normal embryo 551, 2 r. 11 days. Note neural tube, notochord, somites, tail gut ×80.

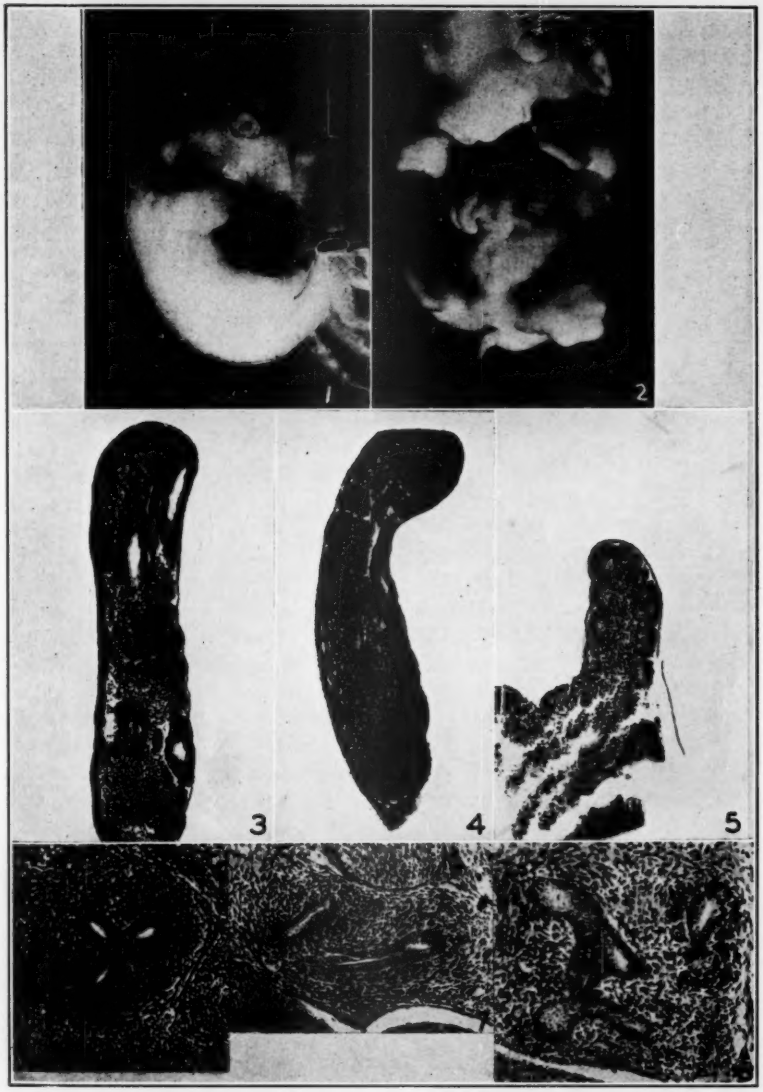
FIG. 4.—Sagittal section of tail of *Sd*/+ embryo. 551, 1 r. littermate of 551, 2 r. Note shrinkage of neural tube, breaking up of notochord, pycnotic granules in somites.

FIG. 5.—Sagittal section of tail of *Sd*/*Sd* embryo. 551, 3 r. littermate of 551, 2 r. Note somites and large number of pycnotic granules all through the tail. ×80.

FIG. 6.—Sagittal section through ureter tip of normal embryo. 5403, 2 r. 12 days. Note branching of tip into pole tubules and darkly stained metanephrogenic tissue around tubules. ×150.

FIG. 7.—Sagittal section through tips of double ureter of *Sd*/+ embryo, 5403, 5 r. littermate of 5403, 2 r. Note complete absence of branching in both primary and secondary ureter. ×150.

FIG. 8.—Sagittal section through urinary system of an *Sd*/*Sd* embryo, 6116, 1 l. 12 days. Below left, blind ending of mesonephric duct; primary and secondary ureter branching off to the right; lower ureter ends blindly, upper ureter branches once, both pole tubules carrying caps of nephrogenic tissue. ×150.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ess takes place in the mesenchyme of the tail with ensuing haemorrhages throughout the tail; subsequently degenerative symptoms in notochord, neural tube, and somites appear. Chromophilic granules, as symptoms of cell pycnosis, were also observed by CHESLEY (1935) in the posterior region of homozygous T/T embryos; however, this condition was not found to antedate other abnormalities. CHESLEY observed the same granules in T heterozygotes. But no cell degeneration as regular and as extensive as that in the *Sd* mutants was found in either short-tailed (T +) or tailless ($T\ t^0$ or $T\ t^1$) mice.

The appearance of haematomata in the abnormal tail seems to be due to a defect of the walls of the blood vessels rather than to any circulatory disturbance. Although abnormalities in the walls were not observed directly, their presence seems very likely, since cell degeneration was observed in all of the other tail structures. If, on the other hand, general circulatory defects were responsible for the breakdown of the tail vessels, one should expect haematomata to appear in the extremities as well, and this was not the case. The breakdown of the blood vessels in the tail does not seem to present an explanation for the abnormal processes in the tail because of the cases in which cell pycnosis was observed in the tail in the absence of haematomata.

A comparison of *Sd* embryos with flexed embryos shows that both mutants have abnormalities of the intervertebral discs, but those in the flexed mice are located in the fibrous part of the disc while in *Sd* mutants the nucleus pulposus (that is, the remnant of the notochord) is abnormal or missing.

Thus, the same end result—namely, abnormalities in the spine and tail—is achieved by different means in the case of these different mutations. This conclusion would not have been possible on the basis of studies of newborn and adult mutants alone. The abnormalities had to be traced back into embryogeny in order to find out the nature of the abnormal processes leading to the malformations. Thus, a fundamental difference appears between the actions of the different tail mutations which at first sight—that is, in the newborn and the adult—looked so very similar. Their similarity consists solely in affecting identical parts of the body, but the chain of events between the gene and the end organ is different in the case of each of these different mutations.

Only one other mutation in mice affecting the urogenital system has been described (BAGG 1925); it brought about absence or underdevelopment and pathological changes of one or both kidneys. Both the pathological anatomy of the abnormal kidneys in newborn and adults as well as the embryogeny of the abnormal organ systems of the BAGG abnormal mice (BROWN 1931) differ from those in the *Sd* mutants. The kidneys of the *Sd* mice differ from normal kidneys mainly in the number of elements; those elements that are present seem normal, while BAGG'S abnormal mice showed definite structural abnormalities of the elements of the kidney.

Our material seems to indicate that it is the ureter which is primarily affected by *Sd* and that the failure of its upper part (the primitive renal pelvis) to differentiate and branch properly, and thus to give rise to a normal excretory system, is responsible for the failure of the secretory system of the kidney to develop normally. Metanephric differentiation was never observed

in the absence of development of the renal pelvis. Differentiation of pole tubules from the primitive renal pelvis on the other hand was always accompanied by corresponding formation of secretory elements in the metanephrogenic tissue. Sometimes the ureter failed to branch off from the mesonephric duct altogether, in which case no metanephric development whatever occurred in spite of the presence of the metanephrogenic tissue. All these facts seem to justify the conclusion that the effect on the ureter precedes that on the metanephros proper. Since no earlier morphological abnormalities were found in the urogenital system of the *Sd* + or *Sd Sd* embryos, it is impossible to state which abnormal processes precede the abnormalities of the ureter.

The simultaneous effect of *Sd* on the axial skeleton and the urogenital system is a phenomenon which cannot be easily explained at the moment. Morphologically and embryologically, the organ systems affected have several properties in common: they are both located in the same region of the body—namely, the posterior region—and they are both of mesodermal origin. But an explanation of the simultaneous effect of the mutation *Sd* based on the common location of the organs concerned would seem doubtful, because other organs in that same region are not affected. The same argument would hold against an explanation based on the common mesodermal origin of axial skeleton and urogenital system since other mesodermal organs are not affected. It seems that in the absence of any knowledge about the primary action of *Sd* and about the normal primary processes in the early development of both axial skeleton and urogenital system, the problem of the pleiotropic effect of *Sd* has to be left open.

Recently, GRÜNEBERG (1943) formulated "two principles and five working postulates as a guide for the developmental analysis of morphological gene effects." The first principle excludes the existence of genuine pleiotropism, a viewpoint which GRÜNEBERG has held and discussed before. GRÜNEBERG's second principle excludes the existence of organ-specific primary gene effects and asserts that the primary action of a gene is either cell-specific or tissue-specific. As GRÜNEBERG argues rightly, it is quite true that our present knowledge permits us to exclude the organ-specific action of genes, since organs represent such composite systems in their chemistry and metabolism that any primary action of the gene on an entire organ may be excluded from consideration. However, we may argue with equal justification against the concept of cell- and tissue-specific action of genes. Cells and tissues represent systems as composite in their chemistry and metabolism as do organs, and genes will not act on them primarily any more than they would on organs. According to our present idea of the action of genes, genes do not act directly on structural elements whether they be organs, tissues or cells, but their primary action concerns physiological processes—that is, not static but dynamic events are affected by them. It seems irrelevant for the problem of primary gene action whether the locus of expression of some particular gene action be cells, tissues, or organs.

We are not any more justified in drawing conclusions from the final result of gene action and calling the gene's primary action cell- or tissue-specific than

we are in calling it organ-specific. In each case, the eventual manner and place of expression of the gene action on morphological structure is far removed from its primary effect. This we can only visualize as affecting fundamental processes which underlie the formation of structure. A distinction between cell- and tissue-specific primary action on the one hand and organ-specific action on the other does not seem justified by the facts of embryology, genetics, or developmental genetics. We know of the interdependence of all parts of the organism during embryogeny, and of the state of flux of the embryo during development; due to this interdependence the process or processes singled out and acted upon by any one gene might find expression in cells or in tissues or in organs or in two or three of them simultaneously. Generalizations such as GRÜNEBERG'S on the basis of his results (rat-lethal, mouse-hydrocephalus) do not seem to be justified. In his particular cases primary gene action might express itself in tissue-specificity (cartilage), but that does not mean that the primary action of the gene in question is tissue-specific or that gene action always is cell- or tissue-specific. The primary action of a gene is probably much more pervasive than is apparent from the gene's visible effects as observed with the crude morphological methods at our disposal. It is to be expected that new and different methods of study will reveal effects of genes overlooked in the first analysis. Furthermore, the eventual effect of a particular gene must depend not only on its own primary action but also on the reactivity of the different parts of the developing embryo and on the particular time at which the gene action takes place. Thus, if the action of a gene expresses itself in abnormalities of one particular tissue, it is conceivable that this tissue at the time was more sensitive to the action of the gene than any other tissue. In such a case one could not call the primary *action* of a gene tissue-specific although its *expression* is tissue-specific. The same argument holds for organs and cells. As to GRÜNEBERG'S concept of pleiotropism—expressed in his first principle which excludes its existence—this has been discussed recently by DOBZHANSKY and HOLZ (1943) who rightly assert the futility of arguing about existence or non-existence of genuine pleiotropism as long as we do not know anything about the primary action of genes.

In our own case, cell- or tissue-specific action of the gene *Sd* are excluded on an equal basis with organ-specific action. The abnormalities of the skeleton and the urogenital system in heterozygous and homozygous *Sd* mice could not be traced back to any common primary tissue- or cell-abnormality. If any common abnormality exists, it must be looked for in some physiological process rather than in a morphological structure. Although no morphological abnormality was found prior to the specific abnormalities of the axial skeleton and urogenital system, it is highly likely that physiological abnormalities do exist before they become manifest morphologically. Before their nature is known it is not possible to decide whether or not these physiological abnormalities are responsible for both the skeletal and the urogenital abnormalities. Although a common morphological basis for the skeletal and urogenital abnormalities can thus be excluded, the question of the pleiotropic action of the *Sd* gene must be left unanswered.

SUMMARY

The development of embryos heterozygous and homozygous for the dominant mutation *Sd* is described.

Two organ systems are affected in these mutants: the axial skeleton and the urogenital system.

The tail of the heterozygotes shows the first abnormalities at the end of ten days after fertilization: cellular degeneration products and small haematomata appear; eventually, the abnormal part of the tail is resorbed.

The tail of the homozygotes shows the first abnormalities at the end of nine days. Again, cellular degeneration products and haematomata are typical symptoms and eventually the entire tail is resorbed.

In both heterozygotes and homozygotes abnormalities of the notochord and neural tube are secondary to the cell degeneration processes in the tail.

The processes leading to the abnormalities of the tail in *Sd* mice differ from those in other known tail mutants.

The urogenital abnormalities of the heterozygotes appear at the age of ten days and consist in that stage in abnormalities of the ureter followed later by abnormalities of the metanephros.

The homozygous embryos show the first urogenital abnormalities around the beginning of ten days after fertilization; the posterior part of the mesonephric duct is missing in most cases, the ureters are short and displaced and frequently doubled. Normal metanephric development never occurs.

From the study of the development of urogenital malformations in heterozygotes and homozygotes it is concluded that the metanephric abnormalities are dependent on those of the ureters.

The problem of the simultaneous effect of *Sd* on axial skeleton and urogenital system is discussed from the developmental point of view.

LITERATURE CITED

BAGG, H. J., 1925 Hereditary abnormalities of the viscera. I. A morphological study with special reference to abnormalities of the kidneys in the descendants of x-rayed mice. Amer. Jour. Anat. **36**: 275–311.

BROWN, ALICE L., 1931 An analysis of the developing metanephros in mouse embryos with abnormal kidneys. Amer. Jour. Anat. **47**: 117–172.

CHESLEY, P., 1935 Development of the short-tailed mutant in the house mouse. J. Exp. Zool. **70**: 429–459.

DOBZHANSKY, TH., and A. M. HOLZ, 1943 A re-examination of the problem of manifold effects of genes in *Drosophila melanogaster*. Genetics **28**: 295–303.

DUNN, L. C., S. GLUECKSOHN-SCHOENHEIMER, and V. BRYSON, 1940 A new mutation in the mouse affecting spinal column and urogenital system. J. Hered. **31**: 343–348.

GLUECKSOHN-SCHOENHEIMER, S., 1938 The development of two tailless mutants in the house mouse. Genetics **23**: 573–584.

1943 The morphological manifestations of a dominant mutation in mice affecting tail and urogenital system. Genetics **28**: 341–348.

GRÜNEBERG, H., 1943 Congenital hydrocephalus in the mouse, a case of spurious pleiotropism. J. Genet. **45**: 1–21.

KAMENOFF, R. J., 1935 Effects of the flexed-tailed gene on the development of the house mouse. J. Morph. **58**: 117–155.

DISTRIBUTION AND GENETICS OF THE COLOR PHASES OF THE RED FOX IN CANADA[1]

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THE colored fox (*Vulpes fulva*) and its geographic races exists throughout most of its range in three main color phases: (1) The red fox is reddish brown to yellow with black markings on the feet and the ears. There is an area of pure white hairs on the mid-ventral line and on the tip of the tail. (2) The silver fox has all the reddish brown hairs replaced by black ones or black with a white band. The width of the band and the distribution of the white-banded hairs determines whether a fox of this phase is a black, one-quarter silver, three-quarters silver, etc. Most of the farmed foxes have been selectively bred so that from three-quarters to the whole of their body is covered with these silvery hairs. The pure white portion of the body remains unchanged. In farmed types such as white faces, ring neck and platinums this white area has been much enlarged. (3) The cross fox is a mixture of the other two types. There is a great deal of variation in foxes of this type. A typical specimen has red flanks, shoulders and cheeks, and silver or black back and neck. The darkened lengthwise line down the back and the dark fur on the neck form a cross from which the animal derives its name. Some foxes are very similar to the red phase but have a smoky appearance because of the number of black hairs scattered through the pelt. These are spoken of in the trade as bastard foxes. Breeding experiments at the HUDSON'S BAY COMPANY fur farm indicate that these foxes are similar to cross fox in their two main coat color factors.

The fox population fluctuates markedly, with peak numbers every ten years in most of its range[1] and every four years in northern Quebec and Labrador. The population has increased over 100 per cent in the past 20 years. The peak years do not coincide in different parts of the country. There is migration from areas of high density, as witnessed by the appearance of runs of northern or western types of fur in places where they are not native.

GENETICS OF THE COLOR PHASES

Early in the history of fur farming it was observed that silvers appeared in the progeny of cross fox parents, and soon a mono-factoral hypothesis was proposed as follows: Red, *SS;* Cross, *Ss;* Silver, *ss.*

Later ILJINA (1934, 1935) proposed a slightly more complicated system with a modifier making different types of cross as follows: Red, *rr;* Smoky red, *Rr dd;* Cross, *Rr Dd;* Cross, *Rr DD;* Silver, *RR.*

As more animals were raised in captivity, it was found that the above

[1] The writer wishes to acknowledge the helpful suggestions and criticisms received from Dr. J. W. MacArthur of the University of Toronto.

schemes would not hold. Cross foxes were often found in the litters of two silver parents. To take care of these exceptions WARWICK and HANSON (1937) proposed a two factor hypothesis. According to their hypothesis the factor combinations for the three phenotypes are:

Red	*Cross*	*Silver*	
AABB red	*AaBB* cross	*AAbb*	*aaBB*
AABb smoky red	*AaBb* blended cross	*Aabb*	*aaBb*
		aabb	

(These five combinations of silver represent different grades of silver.)

POPULATIONS GENETICS

CROSS (1941), at the suggestion of J. W. MACARTHUR, applied the gene frequency method to the HUDSON'S BAY COMPANY fox returns for Ontario. As a result of his analysis he suggests that "a single pair of genes and their alternates govern the color phases of Ontario foxes." Unfortunately, he did not use any test of significance or he would have realized that such a conclusion was unwarranted (see table 1). In fact MACARTHUR pointed out the disagreement in the data at the time and suggested that it was possibly due to pro-

TABLE 1

Test of mono-hybrid inheritance.

PROVINCE	SILVER	CROSS	RED	TOTAL	χ^2
British Columbia	1,662	8,597	10,533	20,792	6.09
	1,653	8,432	10,707		
North West Territories	2,425	21,503	50,899	74,827	23.3
	2,215	21,356	51,256		
Alberta	3,080	20,436	44,965	68,481	169
	2,472	21,085	44,924		
Saskatchewan	2,785	22,909	67,676	93,370	262
	2,101	23,856	67,413		
Manitoba	3,452	23,019	75,237	101,708	973
	2,197	25,468	74,043		
Ontario	3,333	23,668	82,294	109,295	1,063
	1,989	25,553	81,753		
Quebec	3,113	13,883	51,859	68,855	2,919
	1,391	16,787	50,677		

gressive selection against the more valuable silvers, but this is not mentioned in CROSS' paper. ELTON and HALDANE (1942) have both dealt with the fox color types data from the standpoint of selection, and their findings will be discussed in a later section.

The theoretical basis for this study is that the frequency "p" of the dominant gene and the frequency "q" of the recessive gene have mathematical relationships which can be demonstrated for all populations. The monohybrid relationship for foxes would be: reds "p^2," cross "$2pq$," silver "q^2."

Or with the ILJINA scheme it would be:—

red	p^2	$p(p+2y^2q)$
smoky red	$2pqy^2$	
cross	$2(1-y^2)pq$	
silver	q^2	

Where "y" is the frequency of the gene modifier *D* and is found from the equation:

$$y=\sqrt{\left(1-\frac{c}{2(q-s)}\right)}$$ where c = percentage of cross and s = percentage of silver

For a dihybrid relationship we get the more complicated arrangement which is given below:

	BB	*Bb*	*bb*
	p^2	$2pq$	q^2
AA	*AABB*	*AABb*	*AAbb*
	red	smoky red	silver
m^2	p^2m^2	$2(pq)m^2$	m^2q^2
Aa	*AaBB*	*AaBb*	*Aabb*
	cross	cross	silver
$2mn$	$2(mn)p^2$	$4mnpq$	$2(mn)q^2$
aa	*aaBB*	*aaBb*	*aabb*
	silver	silver	silver
n^2	n^2p^2	$2(pq)n^2$	n^2q^2

This gives:	red	$p^2m^2+2(pq)m^2$
	cross	$2(mn)p^2+4mnpq$
	silver	$1-(\text{reds}+\text{cross})$

In the following tables and graphs the above criteria are applied to the HUDSON'S BAY COMPANY fur returns from 1915 to date. The figures, which pertain to more than 700,000 fox pelts, are divided into seven major groups representing the foxes taken in the different provinces. The position of these provinces and the area covered by these fur returns is given in the map, figure 1. The provincial boundary lines are used not because they constitute biological divisions but because they are easily found on the map and serve

as a yardstick to point out sections where a more detailed analysis should be made.

TEST FOR MONO-HYBRID RELATIONSHIP

Applying the $p^2:2pq:q^2$ formula, we get the results given in table 1. In column 5 the significance of the difference between the theoretical and the observed is tested by the chi square. These chi square values show that the only province in which the proportions of the different color phases are consistent with the mono-factoral hypothesis is British Columbia. In this province the P value is 0.11, which means that such a deviation from the theoretical could be expected to occur 11 times in every hundred samples of like

FIG. 1.—Map of Canada showing provinces and area from which fox data were obtained.

size. This fit is close enough that the deviations can be attributed to chance. Thus we may say that the frequencies observed in British Columbia could be caused by a single gene and its allele.

The P value for other provinces is so small that the chances are over 1,000 to 1 against these color segregations being due to a single gene.

The enormous chi square value for Ontario rules out CROSS' explanation of a single factor difference in Ontario colored foxes.

Using the hypothesis proposed by ILJINA, we get a slightly better fit in most cases, but the chi square value is still enormous.

These values are worked from the total of each type for various provinces, and before we can entirely dismiss the single factor hypothesis it is necessary to consider MACARTHUR'S suggestion of progressive selection against recessives.

SELECTION AND CHANGES IN THE PERCENTAGE OF SILVERS

The data presented by ELTON (1942), page 280, show that in Labrador the percentage of silver fell steadily from 16 per cent in 1834–1843 to 5 per cent in 1924–1933. A similar decrease has taken place in western Canada. In 1825–1850 the percentage of silver in British Columbia according to MCTAGGART-COWAN (1938) was 19–25 per cent, and by 1940 it had fallen to less than 7 per cent. A similar though less spectacular drop is noted along the Mackenzie River between 1880 and 1940.

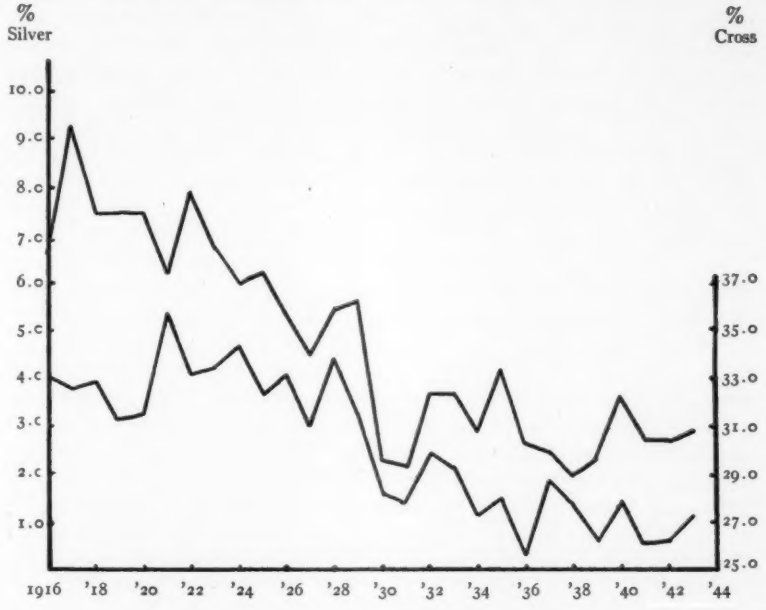

FIG. 2.—Yearly proportion of silver (upper line) and cross foxes in Alberta.

This progressive decrease in silver is still going on in the period under study, as can be seen from figure 2. The two lines on this graph represent the percentage of silver and cross foxes taken at HUDSON'S BAY COMPANY posts in Northern Alberta and portray a more regular and striking decrease than similar figures for other provinces. In order to observe the differences between the provinces, the regression coefficients are given in table 2. This shows that the percentage of silver is decreasing in all provinces, and that in Alberta, where the decrease is highest, the silver proportion gets 0.23 per cent less each year. The percentage of cross is not so uniform in its changes. Four provinces show decreases varying from 0.13 per cent to 0.34 per cent; two

show increases varying from 0.02 per cent to 0.07 per cent; and one is almost stationary with minus 0.003 per cent.

This diminution in the percentage of silver has been commented upon by CROSS (1941) and ELTON (1942), HALDANE has worked over the data published by ELTON. He assumed that the Alaskan gene was absent in the Labrador stock and that ILJINA's hypothesis operated here. He tested the relative fitness of silver to the other color types by the use of his formula: $U_N - U_0 + \log_e U_N - \log_e U_0 = KN$, where $U = r/R$, U_0 is the initial value, U_N the value after N generations. He found for the Labrador fox that $K = .0328$ or that the annual rate of kill of silvers exceeded red and cross by between 3 and 4 per cent. It must be remembered that this selection rate is based on the assumption that the Alaskan gene is not present. Also that the distortion in the monohybrid ratio which would require an inbreeding coefficient of alpha $= .2619$ to satisfy is caused by more silvers being trapped than their normal proportion in the population. To explain the greater proportion of silvers taken, ELTON and HALDANE have to rely on the selective action of shooting, since trapping is not selective. According to their theory, a silver fox, because of the relatively higher value of its pelt, was pursued and shot more arduously than were crosses and reds. This would cause the percentage of silver to drop, and the percentage of cross would also drop because there would be less silver×red and silver×cross fox matings to produce crosses.

We raise three objections to this theory: (1) Information gathered from fur traders shows that for the past thirty years less than 5 per cent of foxes were shot and that these were of all types indicating chance shots rather than pursuit of a particular type. (2) In two provinces the cross fox percentage has increased while that of silver was decreasing. (3) The silver percentage is still decreasing, but the selective incentive has been removed. The farming of silvers has decreased the price and improved the type so much that a wild silver is worth very little more than a red. At the same time the royalty has been maintained in most provinces on the old basis of silver $5.00, cross $1.50,

TABLE 2

Percentage change in the proportion of silver and cross foxes from 1916–1944.

PROVINCE	SILVER		CROSS	
	MEAN	YEARLY CHANGE	MEAN	YEARLY CHANGE
British Columbia	8.0%	−.157	41.3%	+.073
Alberta	4.9%	−.234	30.0%	−.340
N.W.T.	4.0%	−.074	29.0%	−.003
Saskatchewan	3.0%	−.0408	24.6%	+.0204
Manitoba	3.4%	−.093	22.6%	−.129
Ontario	3.24%	−.123	21.0%	−.308
Quebec	5.72%	−.181	20.7%	−.242

red $0.50. Thus at average values a red at $9.00−.50=$8.50, and a silver at $13.00−$5.00=$8.00 puts the silver in the same price bracket as reds or often discounts against them. As a corollary of this royalty/price relationship it is to be expected that classification has become discriminating against silvers and crosses. When any doubt exists about the color phase of a skin it is placed in the lower royalty class.

If because of the aforementioned points we discard selection by man, then we have the following alternatives: (1) Silver foxes are not so prolific, and HALDANE'S K really measures their breeding and survival rate as compared with other types. In favor of this point is the fact that platinums and other mutant types have lower fecundity, and that the decrease in percentage of silver is accompanied by a great increase in population and catch of foxes. (2) The intermingling of the Alaskan and Canadian genes may have had more to do with the decrease in silver than selection by man. In favor of this is the fact that the silvers have decreased at different rates in different provinces (see table 2, 3); also that the decrease in silvers was sometimes accompanied by an increase in cross fox.

In considering these decreases it should be borne in mind that a north-south gradient did and does exist in silver foxes. Silvers are more abundant in the north than in the south. With the northward advance of settlement, foxes with their love of forest clearings have also moved north and increased in number. Evidence of this northward migration is seen in the increasing numbers of red fox that are being taken in the white fox country. Given this mixing of southern types with their lower recessive frequency, the number of silvers in the northern population was bound to decrease. However, there is not enough definite evidence to entirely rule out some selection against the recessive genes, but from a practical standpoint we think any such selection must have been from differential birth or mortality rates between types rather than from selective shooting by man.

In order to eliminate the effects of this decrease in the percentage of cross and silvers on the genetical studies we have arranged the data in groups of five years, and table 3 gives the percentage distribution in the different provinces for each of these pentades.

THE TWO FACTOR HYPOTHESIS

Assuming that the two factor hypothesis of WARWICK and HANSON (1937) is operating in the Canadian populations being studied, we can find the geographic distribution of the Alaskan gene *A a* and the Canadian gene *B b*.

Working with various values of "p" and "m," we have found frequencies of *A* and *B* which fit all ratios, and these are given in the last two columns in table 3. Careful examination of these data show that, with the exception of British Columbia, the proportions of the different types do not satisfy the single factor theory unless inbreeding is postulated. In a few cases the frequency of either gene *A* or *B* reaches 0.98. A frequency of 1.0 would, of course, eliminate the gene from the study. From these data it appears that the lack of disagreement with the monofactoral hypothesis is real and not due to progressive selection.

TABLE 3

Distribution of color phases in Canadian Provinces by Pentades.

	Silver	*Cross*	*Total*	*A*	*B*
		British Columbia			
1916–1925	8.6%	41.0%	4,512	.71	1.0
1926–1930	9.0	43.0	5,489	.70	1.0
1931–1935	7.3	39.4	4,825	.73	1.0
1936–1940	7.8	41.8	4,637	.72	1.0
		Alberta			
1916–1920	8.0%	35.2%	5,331	.75	.90
1921–1925	6.1	33.4	20,885	.78	.90
1926–1930	4.75	32.7	13,725	.80	.90
1931–1935	3.55	28.0	20,214	.83	.90
1936–1940	2.8	27.3	6,198	.83	.97
		North West Territories			
1916–1920	5.4%	29.5%	7,546	.81	.87
1921–1925	3.5	24.9	8,584	.84	.88
1926–1930	4.8	29.6	9,372	.82	.88
1931–1935	3.2	28.8	46,658	.83	.97
1936–1940	3.6	28.7	46,511	.83	.96
		Saskatchewan			
1916–1920	3.80%	22.1%	4,517	.87	.87
1921–1925	3.84	23.7	14,941	.86	.90
1926–1930	3.64	24.7	9,637	.85	.95
1931–1935	2.73	23.1	24,058	.86	.95
1936–1940	2.93	27.3	17,596	.83	.98
		Manitoba			
1916–1920	5.1%	24.6	7,601	.85	.85
1921–1925	4.36	25.4	14,013	.85	.87
1926–1930	3.28	23.3	16,870	.86	.90
1931–1935	2.63	21.7	27,577	.87	.91
1936–1940	3.38	22.7	20,364	.86	.89
		Ontario			
1916–1920	4.4%	23.9%	12,167	.86	.86
1921–1925	3.95	23.8	11,356	.86	.89
1926–1930	3.9	23.4	28,303	.86	.89
1931–1935	2.7	20.5	21,478	.88	.90
1936–1940	1.88	18.0	28,331	.90	.90
		Quebec			
1916–1920	5.9%	22.4%	6,249	.86	.82
1921–1925	7.0	24.5	5,048	.85	.79
1926–1930	5.3	22.2	16,368	.87	.79
1931–1935	5.0	20.2	10,935	.88	.80
1936–1940	3.5	18.3	22,378	.90	.85

GENE A—ALASKAN

The recessive gene *a* which is responsible for silvers is most abundant in the west from which it derives its name. The frequency of *A* in British Columbia is 0.70–0.73 and the recessive falls off rapidly as one moves eastward until *A* reaches a high of 0.85–0.90 in Ontario and Quebec (see fig. 3). The variation in frequency in the different pentades is small, showing no definite trend and re-

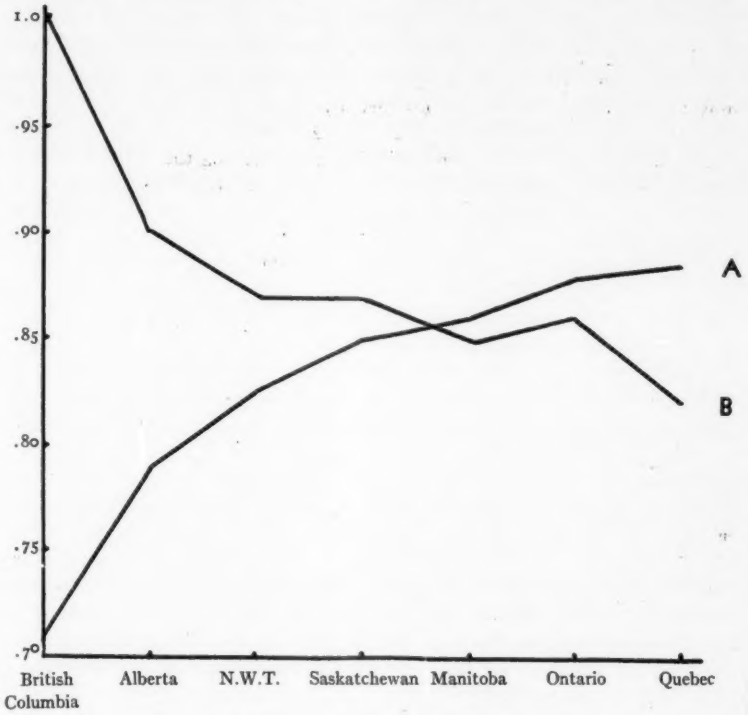

FIG. 3.—The frequencies of genes *A* and *B* by provinces.

quires no explanation for British Columbia, North West Territories, and Manitoba. Saskatchewan shows only a slightly larger amount of variation than the previous three, but it exhibits a definite trend. The frequency of *A* has decreased from 0.87 to 0.83, whereas in the other provinces the gene *A* has increased. This anomaly in Saskatchewan is probably due to the pivotal position of the province with respect to the geographical distribution of frequencies of the genes *A* and *B*. In Ontario and Quebec the gene *A* has increased from 0.85–0.90, and in Alberta it has increased from 0.75–0.84.

GENE B—CANADIAN

The recessive allele of the Canadian gene *B* is most abundant in Quebec and becomes decreasingly abundant as one moves westward (fig. 3). At the far west, in British Columbia, with its isolating mountain chains, the gene is not present at all. The changes in gene frequency in the various pentades are given in table 4. This shows that the greatest change has taken place in Saskatchewan where gene *B* increased from 0.87 to 0.98 at the same time as gene *A* decreased from 0.87 to 0.835. This great change could be expected from Saskatchewan's geographic position in relation to the initial frequency of *A* and *B*. The upsurge of the population cycle usually starts near Lake Athabasca and moves eastward probably accompanied by migration. This eastward migration has led to an increase of gene *a* in Saskatchewan. In the same period Saskatchewan has become more settled, thus increasing the fox habitat. This new habitat has become populated with foxes of the western type.

TABLE 4

Changes in the frequency of Gene B.

	FREQ. IN 1ST PENTADE	CHANGE IN EACH PENTADE				TOTAL DEVIATION
		2ND	3RD	4TH	5TH	
British Columbia	1.0	—	—	—	—	0
Alberta	.9	—	—	—	.07	.07
North West Territories	.87	.01	—	.09	−.01	.09
Saskatchewan	.87	.03	.05	—	.03	.11
Manitoba	.85	.02	.03	.01	−.02	.04
Ontario	.86	.03	—	.01	—	.04
Quebec	.82	−.03	—	.01	.05	.03

The next greatest change took place in the North West Territories. This increase was not accompanied by a compensating decrease in *A*, so the percentage of silver also decreased. In Alberta there was no change in the first four pentades, and then in the fifth pentade there was a sudden increase of 0.07. This increase was accompanied by a population decrease, and it will be interesting to see whether this increase in *B* is a mere fluctuation or a definite trend. It is interesting to note that the largest increases in *B* occur in geographic progression as listed below:

Pentades			
2nd	*3rd*	*4th*	*5th*
Ontario	Manitoba Saskatchewan	North West Territories	Alberta

No change took place in British Columbia, probably due to its isolation. The changes in Manitoba and Ontario are small increases and require no comment here. Quebec figures are erratic and exhibit no particular trend. The decrease in *B* was accompanied by a freak population peak. In this section of the country

we have a four year cycle in the north and a ten year cycle in the south. Southward migrations after northern peak years and northward migrations after southern peak years have so mixed the fur returns for each part of the province that changes over short periods such as five years are not reliable unless they exhibit a definite trend. Silvers exist in the greatest proportion when the cycle is at its lowest ebb, and most of the foxes caught are of the northern type.

DISCUSSION

After studying the changes in frequency of these two genes it appears that a great deal of the changes in the percentage of silver are accounted for by the mixing of the two genes. This is especially true in Saskatchewan and Manitoba, the two central provinces which are located between the high frequency of the Alaskan gene *a* in the west and the Canadian gene *b* in the east. In most provinces, however, there is a progressive decrease in one or other of the silver-producing genes without a concomitant increase in the other gene. Therefore, the percentage silver is getting progressively less at the rates shown in table 2. This decrease is even more apparent when data for the past 100 years is considered instead of just 26 years as in the present paper. If we apply the method of the present study to ELTON'S Labrador data, we find that for the past 30 years the data fit into the general scheme quite consistently, but the earlier data do not. Therefore it appears that although the interrelationships of the genes *A* and *B* explain the ratios and some of the decrease in silvers and cross, there is also some other influence at work selecting against the recessive genes. Some possible agents of selection are: (1) Migration of southern types with their lower silver frequency into the area studied. (2) Differential survival or reproductive rates in the different color phases. (3) Difference between present and past classification of color types. Cross foxes with a predominance of red are now classified as red, "bastards" are also put in with reds, thus reducing the cross class. Wild silvers because of their inferiority to the ranched product and consequent low price are now often utilized for parka trimmings, etc., and do not appear in the fur returns. As more data are collected it should be possible to test each of these points.

SUMMARY

Colored fox exist in three color phases in Canada. The population is increasing, but the proportion of silver and cross is decreasing.

Gene frequency tests indicate that the ratios are consistent with the theory of WARWICK and HANSEN and can be explained by the operation of two factors. The recessive of the Alaskan factor *A* is most abundant in the west and becomes decreasingly abundant in geographic progression as one moves eastward. The Canadian gene *B* has the opposite distribution.

The recessives *a* and *b* are both becoming less abundant. It is suggested that this is due either to the influx of the southern fox with its known lower frequency of *a* and *b* or to continuous selection against the silver type. Such selection must now operate through differential fertility or survival of silver foxes in comparison to reds and crosses.

LITERATURE CITED

COWAN, I. McT., 1938 Geographic distribution of colour phases of the red fox and black bear in the Pacific Northwest. J. Mammal. **19**(2): 202–206.

CROSS, E. C., 1941 Colour phases of the red fox (*Vulpes fulva*) in Ontario. J. Mammal. **22**(1): 25–39.

ELTON, C. S., 1942 Voles, mice, and lemmings. Oxford: Clarendon Press.

HALDANE, J. B. S., 1942 The selective elimination of silver foxes in eastern Canada. J. Genet. **44**: 296–304.

ILJINA, E. D., 1934 Nasledovanie osnovnoiokraski lisic. Zool. Zh. **13**: 701–708.

1935 Osnovy genetiki i selekeil pusnyh zverei. Moscow.

WARWICK, B. L. AND, K. B. HANSON, 1937 Yearbook of Agriculture, U. S. Dept. Agric. **1937**: 1388–1394.

THE RELATION BETWEEN POLLED AND HERMAPHRODITIC CHARACTERS IN DAIRY GOATS

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THE problem of the occurrence of hermaphrodites in dairy goats, especially in purebred herds where attention is given to the breeding of polled animals, has led to inquiries regarding the relation between the polled condition and the birth of hermaphrodites. Several observers have noted the fact that horned hermaphrodites are very rare or perhaps do not occur at all. ADDINGTON and CUNNINGHAM (1935), of the New Mexico Station, in a study of the heredity of horns in goats noted that four hermaphrodites, all polled, appeared in matings between animals heterozygous for the polled condition. PAGET (1943) reported that horned hermaphrodites are a very great rarity, known only in occasional instances among British goats. ASDELL (1944) has observed about 200 intersexual goats both in England and in America and found all to be hornless.

These observations and direct inquiries have led to a study of the polled or horned condition of hermaphrodites and normal animals in the UNITED STATES DEPARTMENT OF AGRICULTURE'S herd of Saanen and Toggenburg goats at Beltsville, Md.

EXPERIMENTAL DATA

The period included in this study covers 20 years from 1925–1944, inclusive. The data recorded include number of polled and horned animals, number of normal males, females, and hermaphrodites, and the various types of matings. These data are shown separately for Saanens and Toggenburgs in table 1. The horned character of several of the progeny was not recorded, and in a few cases sex was not recorded, the latter being stillbirths and abortions. In some instances the horned character of the dams was not known. These have been indicated in the table by a question mark. The bucks in both herds were polled except one of the Toggenburgs, which sired only 11 kids. One Saanen buck evidently was homozygous for the polled condition, since all of the 66 kids which he sired out of both polled and horned does were polled.

All hermaphrodites produced in the Beltsville herd, and their parents, were polled. Horns in goats are inherited as a simple recessive. Therefore, in matings between animals heterozygous for the polled condition, one quarter of the progeny should have horns. In the Saanens the ratio was 219 polled:63 horned; and in the Toggenburgs 255:89. These numbers agree very well with expectation. Chi-square for the two herds combined is 1.203 with a corresponding P value of 0.548. In deriving these values, chi-square is the sum of the chi-squares for each breed calculated separately, and P is caclulated for two degrees of freedom (the sum of one degree of freedom for each breed).

TABLE 1

Types of progeny from different types of matings of Saanen and Toggenburg goats. 1925–1944, inclusive.

TYPE OF MATING*	NUMBER OF DOES	MALES		FEMALES		HERMAPHRODITES	HORNED CHARACTER UNDETERMINED		SEX UNDETERMINED	TOTAL
		P	*p*	*P*	*p*	*P*	MALES	FEMALES		
Saanen										
PP×*Pp*	16	24	—	14	—	12	—	—	—	50
PP×*pp*	6	10	—	6	—	—	—	—	—	16
Pp×*Pp*	55	107	27	64	31	31	15	11	5	291
Pp×*pp*	22	18	15	23	11	—	5	8	2	82
Pp×*Pp* (?)	7	9	3	8	2	—	1	1	3	27
Total	106	168	45	115	44	43	21	20	10	466
Toggenburg										
Pp×*Pp*	62	117	42	76	41	36	7	8	2	329
Pp×*pp*	44	45	52	50	47	—	12	8	2	216
pp×*pp*	2	—	1	—	2	—	—	—	—	3
Pp×*Pp* (?)	7	16	5	10	1	—	1	4	—	37
Pp×*pp* (?)	6	4	4	7	10	—	—	2	—	27
Total	121	182	104	143	101	36	20	22	4	612
Saanen Buck×*Toggenburg Does*										
Pp×*Pp*	1	—	—	1	—	—	—	1	—	2
Pp×*pp*	3	1	2	3	—	—	2	—	—	8
Grand Total	231	351	151	262	145	79	43	43	14	1088

* *PP* represents homozygous polled; *Pp*, heterozygous polled, and *pp*, horned animals.

(?) indicates the horned character of the dam was undetermined, but probably was of the character indicated. *P* and *p* under males, females, and hermaphrodites, indicate polled and horned, respectively.

EATON and SIMMONS (1939) have reported hermaphrodism to be inherited as a simple recessive. If, however, as has been suggested by ASDELL (1944), only females are affected, matings between heterozygous normals will give 7 normals: 1 hermaphrodite. Divided as to sex, this ratio becomes 4 males: 3 females: 1 hermaphrodite. Matings of homozygous recessive bucks with heterozygous does should give 50 per cent males, 25 per cent females, and 25 per cent hermaphrodites. There was one such male in the Saanen breed. The number of hermaphrodites observed in each breed did not deviate significantly from expectation. More detailed analysis of the data will be given in the next section of this paper.

The sex ratio for goats is usually reported with a high excess of males. In the Saanens there were 130.7 males per 100 females and in the Toggenburgs 115.0.

If the hermaphrodites are considered as females, the sex ratio approaches more nearly the 50:50 usually expected for most animals. The sex ratio for both breeds combined then becomes 103.0 males per 100 females, or expressed in percentage, 50.7 per cent males.

GENETIC ANALYSIS OF DATA

Since all, or most hermaphrodites, are hornless, there is the possibility that the genes for hornless and hermaphrodism may be linked. Working on this theory and on the known heredity of horns, a detailed analysis of the data may be attempted.

If there is linkage between the genes for the characters concerned, it may be assumed that *Ph ph* is the formula for heterozygous animals of both sexes, *P* denoting polled, *p* its allele, and *h* and *H* hermaphrodism and normal, respectively. Horned animals of either sex would then be *pH pH* and hermaphrodites *Ph Ph*. If, however, only females are affected by the *h* gene, males of the formula *Ph Ph* would be normal. All polled does according to this scheme are heterozygous and capable of producing hermaphrodites when mated with polled bucks.

There are in each breed in the Beltsville herd family lines based on the relationship of the bucks. These lines are shown in figures 1 and 2. In each of these

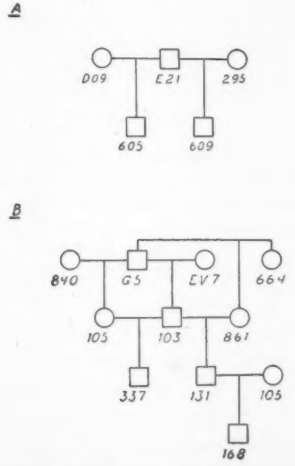

FIG. 1.—Pedigree of two lines of Saanen bucks.—A. Does D09 and 295 were unrelated to E21. —B. 840 was a daughter of 664 and is the only horned doe shown in the pedigree. Neither EV7, 664, nor 840 was related to G5.

lines matings were made to half-sisters, to dams, daughters, and granddaughters of the bucks as well as to unrelated does in an attempt to increase milk production. Only polled bucks were used except in the one case previously mentioned.

The number of progeny from certain bucks or groups of bucks makes possible a rather detailed analysis for the several lines. For convenience in classifying, five classes may be recognized as follows: polled males, horned males, polled females, horned females, and hermaphrodites which are also included in calculating the number of polled animals. For matings between heterozygotes the ratio would be for the five classes named: 3:1:2:1:1. In the case of the homozygous buck in the Saanen breed there would be only three classes, males,

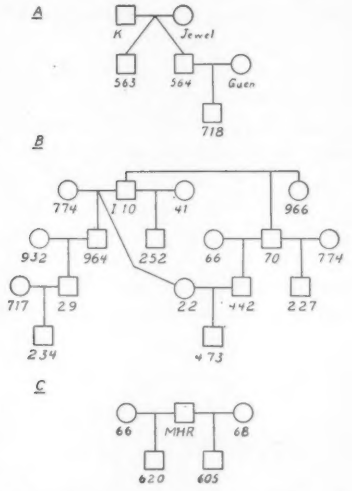

FIG. 2.—Pedigree of three lines of Toggenburg bucks.—A. Neither Jewel nor Guen was related to Buck K.—B. Doe 717 was a full sister to 718 of the previous pedigree. 774 was a daughter of 718×717 of the previous pedigree, and unrelated to 110. 966 was unrelated to 110 and was half-sister to 932. Doe 41 was a daughter of 964×965. 965 was a full sister of 966. 66 was a daughter of 110×717. Does 717, 966, 932, and buck 252 were horned. Buck 227 died and produced no progeny.—C. 66 is the same animal as in pedigree B above. 68 was a daughter of 110×774 in the above pedigree.

females, and hermaphrodites, all polled, in the ratio 2:1:1. The ratio for heterozygous bucks mated with horned does should be 1:1:1:1, there being no hermaphrodites produced. The numbers observed, numbers expected, chi-square and P values for the various lines are shown in table 2. In making these calculations correction to a sex ratio of 50.7 per cent males has been made. For the total value of chi-square, the sums of all the chi-squares were taken, and P was calculated for degrees of freedom equal to the sum of the degrees of freedom for the several groups of similar type matings. P values of 0.05 or higher are considered not significant deviations from the expected class values.

DISCUSSION

The numbers observed in the different classes fit the expected numbers very closely. However, there are a few questions which are not settled. According

TABLE 2

Goodness of fit for observed classes of kids produced by various sires or sire lines in Saanen and Toggenburg goats.

Saanen

	MALES		FEMALES		HERMAPHRODITES	TOTAL
	POLLED	HORNED	POLLED	HORNED		
E21 line×Polled does						
Observed	22	4	8	3	5	42
Expected	15.97	5.32	10.35	5.18	5.18	42
	D.F.=4	χ^2=4.062		P=0.395		
Male 103×Polled does						
Observed	27	7	10	7	8	59
Expected	22.44	7.48	14.54	7.27	7.27	59
	D.F.=4	χ^2=2.458		P=0.649		
Males 131, 168, and 337 ×Polled does						
Observed	18	7	16	5	5	51
Expected	19.39	6.46	12.57	6.29	6.29	51
	D.F.=4	χ^2=1.610		P=0.857		
Other heterozygous bucks×Polled does						
Observed	49	12	38	18	13	130
Expected	49.43	16.48	32.05	16.02	16.02	130
	D.F.=4	χ^2=3.140		P=0.532		
Total for 4 groups	D.F.=16	χ^2=11.270		P=0.792		
Homozygous male G5 ×Polled does						
Observed	24	—	14	—	12	50
Expected	25.35	—	12.33	—	12.32	50
	D.F.=2	χ^2=0.306		P=0.858		
Homozygous male G5 ×Horned does						
Observed	10	—	6	—	—	16
Expected	8.11	—	7.89	—	—	16
	D.F.=1	*χ^2=0.483		P=0.489		
Heterozygous males ×Horned does						
Observed	18	15	23	11	—	67
Expected	16.98	16.98	16.52	16.52	—	67
	D.F.=3	χ^2=4.678		P=0.197		

* Yates' "correction for continuity" was used here. It consists in the subtraction of 0.5 from the deviation from expected for each class.

TABLE 2—(*continued*)

Goodness of fit for observed classes of kids produced by various sires or sire lines in Saanen and Toggenburg goats.

Toggenburg

	MALES		FEMALES		HERMAPH-	
	POLLED	HORNED	POLLED	HORNED	RODITES	TOTAL
K line×Polled does						
Observed	25	9	20	5	4	63
Expected	23.96	7.99	15.33	7.76	7.76	63
	D.F.=4	χ^2=4.263	P=0.369			
Male 110×Polled does						
Observed	28	9	13	9	12	71
Expected	27.00	9.00	17.50	8.75	8.75	71
	D.F.=4	χ^2=2.408	P=0.658			
Males 70 and 964 ×Polled does						
Observed	23	10	10	7	7	57
Expected	21.68	7.22	14.05	7.03	7.02	57
	D.F.=4	χ^2=2.318	P=0.675			
Males 29, 234, 442, and 473×Polled does						
Observed	16	9	13	4	3	45
Expected	17.11	5.70	11.09	5.55	5.55	45
	D.F.=4	χ^2=3.916	P=0.414			
MHR, 605 and 620×Polled does						
Observed	16	4	7	6	4	37
Expected	14.07	4.69	9.12	4.56	4.56	37
	D.F.=4	χ^2=1.382	P=0.844			
Other heterozygous bucks×polled does						
Observed	25	6	23	11	6	71
Expected	27.00	9.00	17.50	8.75	8.75	71
	D.F.=4	χ^2= 4.319	P=0.362			
Total for 6 groups	D.F.=24	χ^2=18.606	P=0.770			
Heterozygous bucks ×horned does						
Observed	49	56	57	57	—	219
Expected	55.52	55.52	53.98	53.98	—	219
	D.F.=3	χ^2=1.108	P=0.774			

TABLE 3

Birth data for normal and hermaphrodite kids in the Beltsville Saanen and Toggenburg goats.

	SAANEN	TOGGENBURG
Number of kids born	466	612
Number of males	234	306
Number of females	179	266
Number of hermaphrodites	43	36
Sex undetermined	10	4
Single hermaphrodites (number of cases)	6	11
Twin hermaphrodites (number of cases)	1*	2*
Male with hermaphrodite (number of cases)	13	10
Two males with hermaphrodite (number of cases)	6	1
Female with hermaphrodite (number of cases)	9	6
Two females with hermaphrodite (number of cases)	2	—
Male and female with hermaphrodite (number of cases)	5	4
Number of births	251	341
Average number of kids per birth	1.86	1.79

* In the Saanan breed this case consisted of two hermaphrodites with a normal female; in the Toggenburgs, one case was twin hermaphrodites; the other, two hermaphrodites with a normal male.

to the theory presented, all polled does are heterozygous and should produce hermaphrodites when mated with heterozygous bucks. There were, however, 35 does in the Saanen and 35 in the Toggenburg breeds which did not produce hermaphrodites. It is evident that some of these which produced only one to three or four kids had not produced enough progeny for a hermaphrodite to appear. The average number of kids per doe among the 54 which produced hermaphrodites was 8.0, while the 70 does which produced none averaged 3.8 kids per doe. If crossing over between the *Ph*- and the *pH*-bearing chromosomes takes place, a new arrangement of the genes, *PH* and *ph*, will be formed. Polled bucks or does carrying these new combinations (*PH*/*PH* or *PH*/*ph*) should produce polled kids, none of which would be hermaphrodites. The type *PH ph* is a heterozygote and phenotypically indistinguishable from the *Ph pH* heterozygote, but should breed differently. One-half of the horned progeny from *PH ph*×*PH ph* matings should be males, the other half horned hermaphrodites, the double recessive combination. Also, horned does of the type *pH ph* mated to *PH ph* or *Ph ph* bucks should produce horned hermaphrodites. Since all the hermaphrodites observed have been invariably polled, not only in the Beltsville herd, but apparently in goats in general, the theory of linkage, except perhaps for absolutely complete linkage, may be ruled out.

This leads then to the consideration that a single gene may be responsible for both the polled and hermaphroditic characters. Hermaphrodites are supposed to be due to excessive or delayed hormone secretions during or immediately prior to sexual differentiation. It may be assumed that horn growth or development is inhibited by the same hormone concerned with sexual differentiation. While horns are not manifest at this early period, their anlagen could well be determined. Since *P* determines the polled condition, it could also be as-

sumed that a single dose of *P* is not sufficient to disturb normal sexual development, but a double dose of *P*, (*PP*), modifies sexual differentiation as well as inhibits horn development. By this theory, the same ratios are derived as by the two-factor theory. The difference is that two seemingly unrelated characters, one dominant, the other apparently recessive, are produced by a single dominant gene which has a recessive effect in regard to one character, hermaphrodism.

Another question concerns the fertility of homozygous bucks. Why should not these bucks be so modified sexually that they are infertile or physically incapable of breeding the same as similar females? ASDELL (1944) proposed that sex modification in mammals is almost always from female to male. This is due presumably to an excess of male hormone secreted, either by the developing embryo or by the dam, during the period of sexual differentiation of the embryo. GREEN (1942) has shown that an excess of androgen (male hormones) has little effect on the male rat embryo. The effect is manifested by an acceleration in the development of the prostate and seminal vesicles and an increase in the size of the penis. These changes would not seem great enough to affect the fertility of a buck or his ability to perform the sexual act. OTT (1937), however, believed that infertility in bucks in Bavaria was related to hermaphrodism. He reported several cases where the infertile bucks were full brothers to hermaphrodites. He did not state if these were born in the same season, but from the number of cases cited it seems obvious that some of them were not. There are no records in the Beltsville herd to indicate that brothers or sisters of hermaphrodites were infertile.

There were some cases in the Beltsville herd which were questionable. In an earlier report EATON (1943) considered them as grades of the intersexual condition. In the present report, however, they have been regarded as males, since they possessed none of the distinctly female structures found in other cases. There were three such cases which have not been counted as hermaphrodites in this paper. Two of them, cases 20 and 27 of the above mentioned report, were distinctly cryptorchids, with one or both testes undescended, apparently normal males in every other respect. Case 2, which had difficulty in unsheathing and erecting the penis and had small testicles, though pendant in the scrotum, probably should be considered a normal male so far as the gene for intersexuality is concerned. Case 19 which had the unicorn uterus and the structure reported as an ovotestis, is doubtful. This animal was horned and was out of a horned doe. She appeared externally like a normal female, and it was only through dissection for another purpose that the abnormal sex organs were found. The structure called an ovotestis was very unlike the structures terminating the uterine horn in any of the other cases examined, being much smaller and showing none of the characteristics of a testis externally such as globus major, glogus minor, and the vas epididymis. Sections of other testes uniformly showed the presence of seminiferous and efferent tubules. None of these were present in sections of case 19. What was interpreted as rete canals might have been only loose vascular tissue. The location of the follicle was in

the cortical portion of the organ, agreeing with the location in two other observed cases of ovotestes where seminiferous tubules were present also. This case was considered a female in the present paper.

By the injection of estrogen into the pregnant rat, GREENE (1942) produced hypospadiac males and females and also males with undescended testes. The latter, however, possessed other abnormalities of the male sex organs as well as certain female organs which were present. These facts suggest that hypospadias, cryptorchidism, and hermaphrodism may be effects of the same gene. Experimental results with rats seem to indicate that two separate hormones are involved. However, the gene that controls the excessive secretion of androgens might also control an excessive secretion of estrogens, if excessive secretions of these substances are involved. It is probably necessary to take a time factor into consideration also.

The heredity of cryptorchidism in Angora goats seems to resemble somewhat the heredity of hermaphrodism. LUSH, JONES, and DAMERON (1930) reported aberrant sex ratios of 93.8 males per 100 females among 2,358 Angora goats. When 63 cryptorchids were included, the ratio became 99.2:100. Among progeny of cryptorchid sires the sex ratio was 76.6:100 for 807 animals. When the cryptorchids were inculded, the sex ratio was 104.8 per 100. The abovenamed authors and WARWICK (1934) proposed a two-factor recessive for the heredity of cryptorchidism, but the number of cryptorchid progeny from cryptorchid bucks was 13.75 per cent, which differs only slightly from the 12.5 per cent expected for hermaphrodites from heterozygous bucks and does. If the three abnormalities, hypospadias, cryptorchidism, and hermaphrodism, are closely related as suggested by experiments with rats, some homozygous males should be incapable of reproduction, either because of infertility due to cryptorchidism in case both testes are involved, or because of mechanical inability to perform the sexual act through the hypospadiac modification of the penis. However, the cryptorchids found in goats, horses, swine, dogs, and other animals may be due to other genes than those concerned in the production of hermaphrodites, since other abnormalities of the reproductive organs are not usually reported in conjunction with them.

The sex ratio for goats is usually reported with a high preponderance of males. HOWLAND (1924) gave the ratio as 139 males per 100 females, while ADDINGTON and CUNNINGHAM (1935) reported 115 males per 100 females. PAGET's data (1943) gave a ratio of 184 per 100. Omitting the hermaphrodites, the ratio for the Beltsville herd was 121.1 per 100 females. By considering the hermaphrodites as females, the sex ratio approached more closely the theoretical 50:50 and agrees fairly well with the sex ratio for other farm animals. In PAGET's data, however, the sex ratio remained high (123:100), even when the hermaphrodites were counted as females. The results, however, suggest that a large proportion of the hermaphrodites must be females genetically.

PAGET (1943) found in his data that there were no cases of single, twin, or triplet hermaphrodites, but they occurred with a normal male, a normal female, or with both. In the Beltsville data there were 17 cases of single her-

maphrodities and three cases where two were born together, one case of twins, one of two hermaphrodites with a normal female, the other two hermaphrodites with a normal male. The data for the Beltsville herd is given in table 3.

The matings in the Beltsville goat herd were made solely on the basis of milk production records. The occurrence of horns and hermaphrodites was merely incidental, except that polled bucks from high-producing dams were selected for the herd sires. The close agreement of the observed number of horned and hermaphrodite animals with those expected by the linkage theory proposed, leads one to believe that we are on the right road to an understanding of the heredity of these characters and the relation between them. It is possible that there may be a low percentage of crossing over to account for some does which appear not to produce hermaphrodites and to explain the very rare occurrence of horned hermaphrodites if they occur at all. If the proposed genetic theory is correct, it should be fairly easy to eliminate hermaphrodism from the goat herd. Matings of polled×polled will produce hermaphrodites unless some of the animals are crossovers. Mating polled×horned of either sex will give no hermaphrodites, but one-half of the progeny will be polled and one-half horned. Matings of horned by horned should give no hermaphrodites, but all the progeny will be horned. Only in the case of crossovers will hermaphrodites appear from matings in which one or both parents are horned. From the data presented and the observations of others, such a probability is extremely small. Goat breeders, then should not focus their attention on the elimination of horns from the herd if they desire to eliminate the more undesirable character, hermaphrodism.

SUMMARY

Data are presented which show the frequency of the occurrence of horns and hermaphrodites in goats of the Saanen and Toggenburg breeds.

The data, and observations of others, suggest linkage between the genes for polled and hermaphrodism.

The formula *Ph ph* is proposed for heterozygous animals of both sexes. A horned animal would be *ph ph*. *P* designates the polled character; *h*, hermaphrodism. An alternate theory of a single dominant gene with recessive effect as regards hermaphrodism is proposed.

The large excess of males reported for goats suggests that a large proportion of the hermaphrodites must be females genetically. When hermaphrodites were counted as females in the data presented, the sex ratio was 103.0 males per 100 females.

Based on the theories suggested, matings between heterozygous polled animals should produce a ratio of 3 polled males: 1 horned male: 2 polled does: 1 horned doe: 1 polled hermaphrodite. The observed numbers agreed well with the expected.

It is suggested that in order to eliminate hermaphrodism from goat herds, matings should be made between a polled and a horned animal, thus giving one-half of the progeny horned, but with no hermaphrodism appearing.

LITERATURE CITED

ADDINGTON, L. H., and O. C. CUNNINGHAM, 1935 Milk goat breeding. New Mex. Agric. Expt. Sta. Bull. **229**.

ASDELL, S. A., 1944 The genetic sex of intersexual goats and a probable linkage with the gene for hornlessness. Science **99**: 124.

EATON, O. N., 1943 An anatomical study of hermaphrodism in goats. Amer. J. Vet. Res. **4**: 333–343.

EATON, O. N., and V. L. SIMMONS, 1939 Hermaphrodism in milk goats. J. Hered. **30**: 261–266.

GREENE, R. R., 1942 Hormonal factors in sex inversion: the effects of sex hormones on embryonic sexual structures of the rat. Biol. Symp. **9**: 105–123.

HOWLAND, W., 1924 Relative sex frequency of issue from pure Toggenburg goats. Goat World **9**: 3–4.

LUSH, J. L., J. M. JONES, and W. H. DAMERON, 1930 The inheritance of cryptorchidism in goats. Texas Agric. Expt. Sta. Bull. **407**.

OTT, F., 1937 Über die erbliche Bedingtheit der Unfruchtbarkeit der Ziegenlammböcke. Züchtungskunde **12**: 460–468.

PAGET, R. F., 1943 Hermaphrodites. Brit. Goat Soc. Monthly J. **36**: 57–59.

WARWICK, B. L., 1943 Inheritance of the ridgling character in goats. Texas Agric. Expt. Sta. Ann. Report: 28–29.

STUDIES OF EYE PIGMENTS OF DROSOPHILA

II. EFFECT OF TEMPERATURE ON THE RED AND BROWN PIGMENTS IN THE MUTANT BLOOD (w^{bl})[1]

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THE red and brown eye pigments of *Drosophila melanogaster* are different in their properties and developmental history. The formation of the brown pigment depends on diffusible substances derived from tryptophane; that of the red pigment apparently does not. Hence, at least the final stages of the reaction chains leading to the formation of these pigments follow different paths. However, some mutant genes inhibit to various degrees the development of both pigments, and this suggests the existence of a common step in the two sequences of reactions. The nature of this common step is unknown. Its understanding would further clarify the mechanism of pigment formation and of its genetic control.

One might expect that a study of the two pigments in a mutant whose phenotype is affected by environmental conditions would throw some light on their relationship. The mutant blood (w^{bl}—a member of the series of multiple alleles at the white locus) shows such a dependence on temperature. Its eye color is pale brownish yellow at 30°C, and deep reddish purple at 17°C, with intermediates at temperatures between these two extremes. The primary purpose of the work reported here was to (1) determine the period of maximum sensitivity to temperature for each of the pigment components, and (2) describe in quantitative terms their variation with temperature. In the course of the investigation the question arose as to whether the changes with temperature are purely quantitative, and this has led us to (3) examine the absorption spectra of the pigments contained in the eyes of flies raised at different temperatures.

MATERIAL AND METHODS

The desirability of extracting pigment from flies having only one of the components has been pointed out in the first paper of this series dealing with the techniques of pigment extraction and measurement (EPHRUSSI and HEROLD 1944). Prior to this investigation two double recessive stocks were therefore prepared: blood scarlet ($w^{bl};st$) and blood brown ($w^{bl};bw$), the first of these serving for the extraction of red, the second for the extraction of brown pigment. In only one case a pure w^{bl} stock, containing both pigment components, was used.

Unless otherwise stated, the techniques of raising the flies and of pigment extraction used in this work are those described in the above mentioned paper. Owing to the reduction of pigment content in the flies studied here as com-

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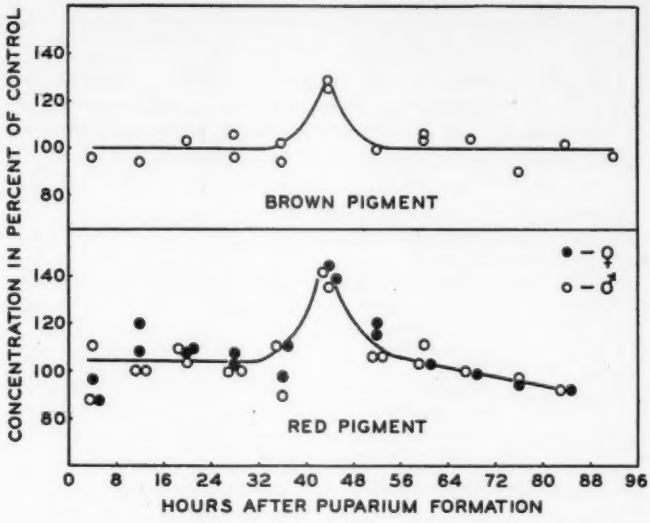

FIG. 1.—Relative concentration of pigment extracted from w^{bl};*bw* (above) and w^{bl};*st* (below) flies subjected to cold treatment at various stages of pupal development (Pulfrich, 470 mμ).

pared with that of pure *st* and *bw* stocks, the number of heads extracted was increased in the different experiments so as to give pigment concentration most favorable for the photometric measurements. The measurements were made with a Pulfrich photometer at 470 mμ. Spectral absorption curves were obtained with a Coleman spectrophotometer.

THE TEMPERATURE EFFECTIVE PERIOD

EPHRUSSI and AUGER (1938) have shown that the pigmentation of the eyes of the mutant w^{bl} is not affected by temperature changes during larval development. The pupa is sensitive to these changes apparently at all stages, but the sensitivity has a peak in the second quarter of pupal development. However, the work of EPHRUSSI and AUGER did not take into account the existence in the eyes of Drosophila of two distinct pigment components and hence provides no answer to the question raised here as to the comparative behavior of the two pigments with respect to temperature.

Our experiments were conducted as follows. Large numbers of well-fed females were allowed to lay eggs over a 20-hour period at 25°C. Larvae were collected over a four-hour period and raised under "standard conditions" at 25° until pupation. Pupation occurs at 25° on the fourth day after hatching of the larva from the egg. At that time the bottles were inspected every hour and only pupae with white puparia were collected, this color indicating that the age of the pupae does not exceed one hour from puparium formation. The pupae were placed in vials, on filter paper moistened with Ringer solution.

For each eight hour period of the four days of pupal life a different group of pupae was subjected to 17°C. Before and after this "cold treatment" the pupae remained at 25°. The controls received no cold treatment.

Just prior to emergence, the pupae were transferred to vials containing the standard food. The imagoes were "aged" for four days prior to extraction. In the *w^bl;st* experiments heads of males and females were extracted separately. Only male heads were extracted in the *w^bl;bw* experiments.

TABLE 1

Effect of cold treatment at different developmental stages of w^bl; st. The treatment period is given in hours from puparium formation. N = number of heads extracted. E = extinction calculated per 10 heads in 1 cc of solvent. % = pigment content calculated in per cent of control. (Pulfrich, 470 mμ).

TREATMENT PERIOD	♂♂ N	♂♂ VOLUME (IN CC) OF SOLVENT	♂♂ E	♂♂ %	♀♀ N	♀♀ VOLUME (IN CC) OF SOLVENT	♀♀ E	♀♀ %
			Experiment 1					
0–8	77	3	0.066	88	98	3	0.067	88
8–16	100	3	0.075	100	94	3	0.090	120
16–24	100	3	0.083	110	67	3	0.081	108
24–32	100	3	0.075	100	73	3	0.078	104
32–40	102	3	0.068	90	80	3	0.084	112
40–48	110	3	0.102	136	100	3	0.107	143
48–56	101	3	0.080	106	58	3	0.090	120
56–64	83	3	0.077	103	114	3	0.077	103
64–72	118	3	0.074	100	77	3	0.075	100
72–80	100	3	0.073	98	34	2	0.072	96
80–88	64	3	0.069	93	101	3	0.069	93
Control	106	3	0.074	100	148	3	0.075	100
			Experiment 2					
0–8	95	3	0.069	111	95	3	0.060	97
8–16	101	3	0.062	100	46	2	0.067	108
16–24	92	3	0.065	105	66	3	0.068	109
24–32	104	3	0.062	100	80	3	0.066	106
32–40	92	3	0.069	111	104	3	0.061	98
40–48	122	3	0.088	142	57	3	0.088	142
48–56	77	3	0.066	106	65	3	0.072	116
56–64	97	3	0.069	111	—	—	—	—
Control	94	3	0.062	100	68	3	0.062	100

The results of four experiments are summarized in tables 1 and 2 and figure 1. In the latter the relative concentrations are given in percentage of the pigment content of untreated control flies, and the experimental points are placed in the middle of the eight hour treatment period.

It can be seen from these results that both pigments show a considerable increase during the period extending from 40 to 48 hours counted from puparium formation. Minor increases of pigment content caused by the short cold treat-

TABLE 2

Effect of cold treatment at different developmental stages of w^{bl};bw. The treatment period is given in hours from puparium formation. N = number of heads extracted. E = extinction calculated per 10 heads in 1 cc of solvent. % = pigment content calculated in per cent of control. (Pulfrich, 470 mμ).

TREATMENT PERIOD	EXPERIMENT 1				EXPERIMENT 2			
	N	VOLUME (IN CC) OF SOLVENT	E	%	N	VOLUME (IN CC) OF SOLVENT	E	%
0–8	44	5	0.162	96	—	—	—	—
8–16	60	5	0.160	94	—	—	—	—
16–24	60	5	0.173	103	—	—	—	—
24–32	45	5	0.163	96	60	5	0.172	106
32–40	60	5	0.172	102	60	5	0.149	94
40–48	60	5	0.210	124	60	5	0.207	128
48–56	60	5	0.167	99	—	—	—	—
56–64	60	5	0.179	106	62	5	0.168	104
64–72	60	5	0.176	104	—	—	—	—
72–80	43	5	0.156	90	—	—	—	—
80–88	60	5	0.172	102	—	—	—	—
88–96	60	5	0.164	97	—	—	—	—
Control	60	5	0.169	100	64	5	0.162	100

ment during other periods of pupal development, if present, are obscured by the variations of the values obtained.

VARIATION OF PIGMENT CONTENT WITH TEMPERATURE

Since temperature affects the eye color of both w^{bl};*st* and w^{bl};*bw* flies only in the pupal stage, in the experiments to be described below larvae of the two stocks were raised at 25° until puparium formation. The pupae were placed in incubators at different temperatures: 17°, 20°, 25° and in some experiments 30°C. They remained at these temperatures until emergence of the flies, and the latter were "aged" at the same temperatures. The time allowed for ageing was five to six days at 25° TND 30°, eight days at 20° and 12 days at 17° (on the assumption of a temperature coefficient = 3). Only male heads were extracted.

The results of four experiments are given in table 3. It can be seen that, in so far as absolute values are concerned, there are considerable differences between the two experiments on w^{bl};*st*, and on w^{bl};*bw*, whether the data are corrected by the weight of flies or not. These differences are in all probability due to differences in the media prepared at different times (see discussion in our first paper). However, the general character of the variation of pigment content with temperature, as measured here, is the same within each pair of experiments. Figure 2, representing the results of one of the experiments, shows that the variation with temperature of the brown pigment follows closely a straight line, while that of the red pigment follows a curve of a strikingly different shape. Both curves show an increase with decreasing temperature.

That the particular shape of the curve for red pigment is due to the presence

of the mutant gene w^{bl} is obvious from the comparison of the curve of figure 2 with that of figure 12 in our first paper. Additional evidence is provided by a single experiment where the red pigment was extracted from the mutant w^{bl}

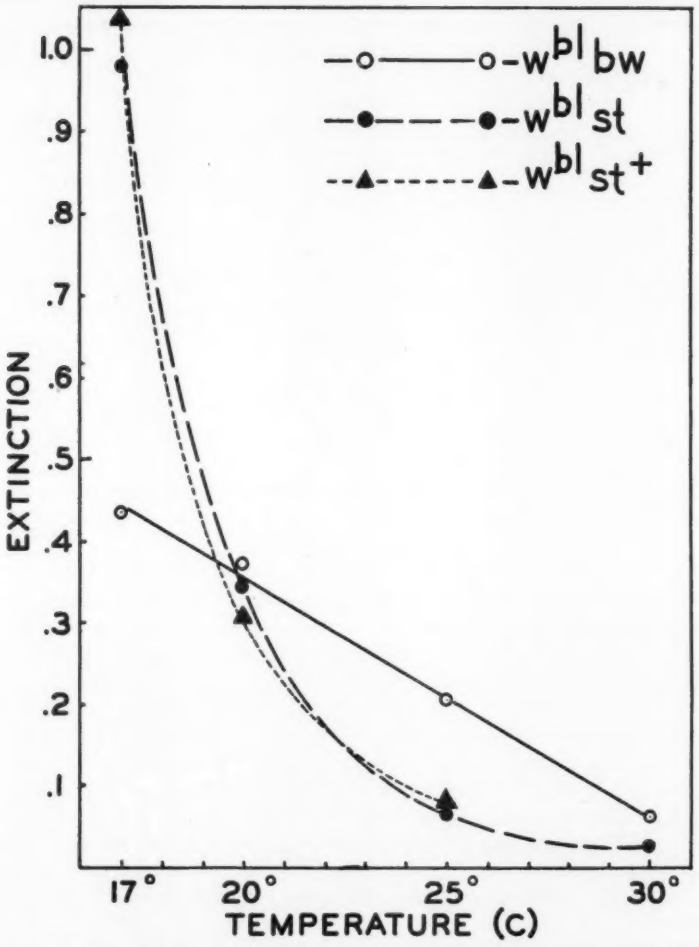

FIG. 2.—Light absorption by extracts from w^{bl};*bw* (brown pigment), w^{bl};*st*, and w^{bl} (red pigment) flies raised at different temperatures (Pulfrich, 470 mμ).

(homozygous for the wild type allele of *st*). The results of this experiment are given at the bottom of table 3 and by the third curve of figure 2. This curve is similar to that given by w^{bl};*st*.

Whether the variations of the brown pigment in w^{bl};*bw* are different from

those found in *bw* within the same temperature range is more difficult to judge. Figure 12 of our first paper shows that the pigment content in the mutant *bw* also decreases somewhat with increasing temperature and that this variation is probably linear. Our data are not numerous enough for the calculation of regression coefficients, but a comparison of the absolute values shows that the variation with temperature of the extinction per head and per degree C is greater in w^{bl};*bw* than in *bw*. However, it will be seen from the considerations presented in the next section that some doubt is cast on the value of quantitative comparisons.

ABSORPTION SPECTRA OF THE EXTRACTS

The data discussed in the preceding section and illustrated by figure 2 reflect the picture of quantitative changes in pigment content only so long as it can be

TABLE 3

Light absorption by extracts of eyes of w^{bl};bw, w^{bl};st and w^{bl} ♂♂ raised at different temperatures. T = temperature. E = extinction calculated per 10 heads in 1 cc of solvent. Wt = average weight of one male in mgs. $E_{corr.}$ = extinction corrected by weight. Aver. = average extinction of different samples corrected as above. N = number of heads extracted. (Pulfrich, 470 mμ).

GENOTYPE	EXPERIMENT 1						EXPERIMENT 2				
	T	*N*	*E*	WT.	$E_{corr.}$	AVER.	*N*	*E*	WT.	$E_{corr.}$	AVER.
w^{bl};*bw*	30	81	0.052	0.846	0.061	0.061					
"	25	50	0.192	0.905	0.212		56	0.162	1.004	0.161	0.160
"	25	39	0.182	0.878	0.207	0.207	55	0.160	1.004	0.159	
"	25	39	0.174	0.878	0.198						
"	20	58	0.318	0.883	0.360	0.371	45	0.287	0.925	0.310	0.315
"	20	62	0.337	0.884	0.381		46	0.296	0.925	0.320	
"	17	45	0.348	0.820	0.424		45	0.369	0.940	0.393	0.391
"	17	50	0.377	0.861	0.438	0.436	39	0.365	0.940	0.388	
"	17	45	0.385	0.861	0.447						
w^{bl};*st*	30	118	0.0264	0.963	0.0274	0.0274	173	0.0208	0.925	0.0225	
"	25	168	0.0672	0.976	0.0689	0.065	262	0.056	0.992	0.0565	
"	25	168	0.0584	0.956	0.0611						
"	20	70	0.3528	1.019	0.3462	0.345	141	0.2744	0.990	0.277	
"	20	70	0.3332	0.968	0.3442						
"	17	64	0.868	1.004	0.865	0.980	118	0.752	0.919	0.818	
"	17	63	0.924	0.844	1.095						
w^{bl}	25	145	0.080	0.986	0.081						
"	20	90	0.322	1.035	0.311						
"	17	115	0.925	0.895	1.034						

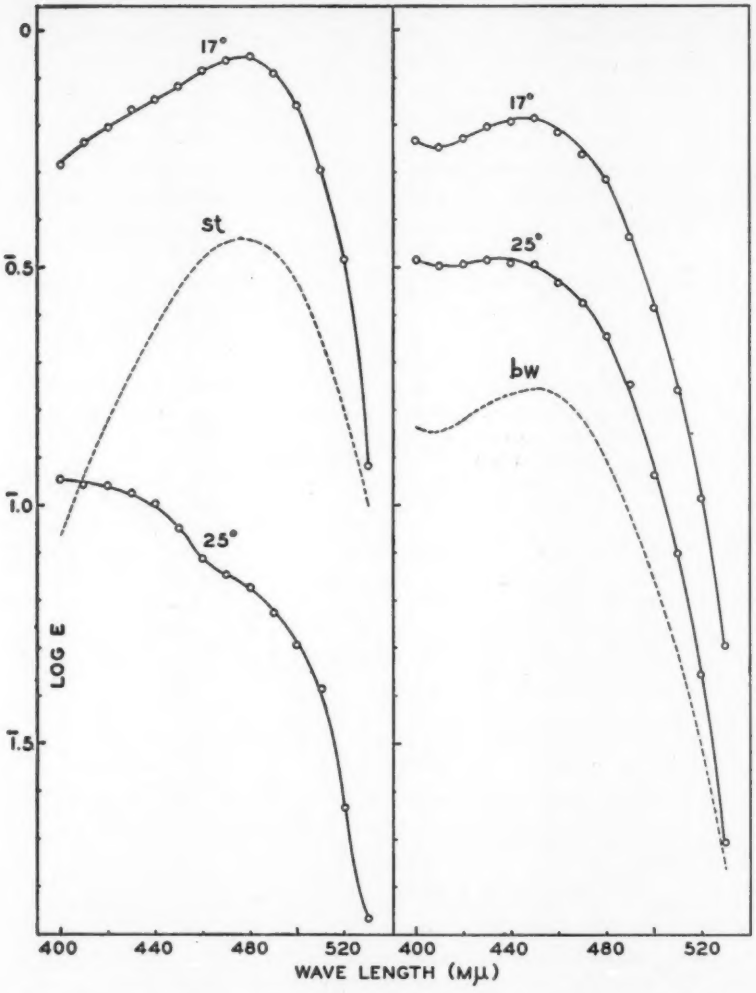

FIG. 3.—Absorption curves of extracts from w^{bl};*bw* (right) and w^{bl};*st* (left) flies, raised at 17° and 25°C. Broken lines—absorption curves of extracts from *st* and *bw* eyes (Coleman).

assumed that there is no change in the quality of the pigments extracted. Two experiments were performed in order to test whether this was actually the case, and the results of one of these are given in figure 3, where *log E* is plotted against wave length. Under these conditions similar pigments give parallel curves independently of concentration differences.

The figure clearly shows that neither the red nor the brown pigment extracts

from flies homozygous for the gene w^{bl} give absorption spectra identical with those obtained from the mutants *st* and *bw* respectively. Moreover, the shapes of the absorption curves undergo changes depending on the temperature at which the extracted flies were raised. The changes in shape of the curves are much more drastic in the case of the red pigment.

DISCUSSION

Although the red and brown pigments of the eye of Drosophila differ markedly in their known chemical and physiological characteristics, it has been suggested that they may be closely related in the unknown early phases of their formation. This suspicion is strengthened by the demonstration reported here that, in the temperature-sensitive mutant w^{bl}, maximal variations of both the red and the brown pigments are produced by temperature changes during the same developmental stage (within eight hours). This is particularly striking in view of the fact that (in the eyes of wild type) at 25°C the deposition of brown pigment begins forty-eight hours after puparium formation, while the red pigment does not appear until much later (about seventy hours).

The relationship between the two pigments could be thought of in terms of a competition of two chromogens for the same substrate, in a manner similar to that proposed by LAWRENCE and PRICE (1940) in the case of anthocyanins and anthoxanthins in plants. Evidence for the existence of a common substrate in the eye pigments of Drosophila has recently been reviewed by EPHRUSSI (1942). It may be recalled in this connection that MAINX (1938) has suggested that these pigments are bound, *in vivo*, to a protein carrier and that BECKER'S (1942) recent chemical study of several Ommatins (a pigment group which includes the brown pigment of Drosophila) has confirmed this view.

However, it has been shown in our first paper that the genes *st* and *bw*, in suppressing one of the pigments, do not increase the amount of the other. This lends no support to the idea of a competition at or after the stage of pigmentogenesis in which the genes *st* and *bw* are operative. A competition at an earlier stage is not excluded.

It was thought at the outset of this investigation that the study of the variations of the phenotype of w^{bl} under the influence of temperature might reveal a competitive relationship of this sort, that is the increase of the amount of one of the pigments at the expense of the other. The data reported above do not support this interpretation. Although precise quantitative comparisons are impossible on account of the changes of the spectra of both the red and brown pigment extracts, there is but little doubt that the quantity of both pigments varies in the same direction as a function of temperature. The immediate effect of temperature therefore must be on the total amount of pigment precursor (s), or on one of its constituent parts available for the formation of pigment, rather than on the shares of the precursor which will be converted to brown versus red pigment.

The fact that the mutant w^{bl} "sensitizes" to temperature both he red and brown pigment systems further emphasizes the idea of a common step in the two reaction chains. Conclusions concerning the rates of change in quantity of

the two pigments cannot be drawn on account of the observed modifications of the absorption spectra. The nature of these modifications will be discussed in the next paper of this series.

SUMMARY

The variation with temperature of the brown and red pigments in the eyes of the mutant w^{bl} was studied.

Cold treatment during different stages of pupal development shows that the period of greatest sensitivity, as revealed by the increase in the amount of both pigments, occurs between 40 and 48 hours after puparium formation. The concomitant variation of the two pigments suggests their close relationship.

The absolute amounts of both pigment components seem to increase at lower temperatures. The rates of change of the two pigments could not be ascertained, for the pigment extracts obtained from flies raised at different temperatures give different absorption spectra.

The existence of a common step in the chains of reactions leading to the formation of red and brown pigment is discussed in the light of these results.

ACKNOWLEDGMENTS

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LITERATURE CITED

BECKER, E., 1942 Über Eigenschaften, Verbreitung und die genetisch-entwicklungsphysiologische Bedeutung der Pigmente der Ommatin- und Ommingruppe (Ommochrome) bei den Arthropoden. Z.i.A.V. **80**: 157–204.

EPHRUSSI, B., 1942 Analysis of eye color differentiation in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **10**: 40–48.

EPHRUSSI, B., and S. AUGER, 1938 Développement des couleurs des yeux chez la Drosophile: effets de la température sur le mutant *blood*. Trav. Sta. Zool. Wimereux, **13**: 279–286.

EPHRUSSI, B., and J. LANE HEROLD, 1944 Studies of eye pigments of Drosophila. I. Methods of extraction and quantitative estimation of the pigment components. Genetics, **29**: 148–175.

LAWRENCE, W. J. C., and J. P. PRICE, 1940 The genetics and chemistry of flower colour variation. Biol. Rev. **15**: 35–58.

MAINX, F., 1938 Analyse der Genwirkung durch Faktorenkombination. Versuche mit den Augenfarbenfaktoren von *Drosophila melanogaster*. Z.i.A.V. **75**: 256–276.

STUDIES OF EYE PIGMENTS OF DROSOPHILA

III. THE HETEROGENEITY OF THE "RED PIGMENT" AS REVEALED BY THE EFFECTS OF THE "WHITE" ALLELES AND BY THE COLOR CHANGES DURING DEVELOPMENT[1]

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THE series of multiple alleles at the white locus (1–1.5) of *Drosophila melanogaster* offers excellent material for studies in physiological genetics. Indeed, this material has been used in investigations of several problems, among which the nature of allelism and the mechanism of dominance can be quoted as examples (review in STERN 1930). All these investigations were made by means of visual observation. However, one of the chief advantages of the locus in question resides in the fact that its focal effect is on pigmentation, a character which should lend itself to more accurate methods of analysis. The application of such methods may be expected to permit further insight into the nature of the multiple changes at the locus and of the processes it controls. The present work is a first step in this direction.

All known mutants at the white locus strongly decrease the pigmentation of the eyes of the adult fly. It can easily be shown (MAINX 1938) that the decrease is due to changes in both the red and brown pigment components. The experiments described in this paper are concerned with the nature of

TABLE I

Light absorption by extracts of eyes of mutants of the "white" series (♀ ♀, homozygous for st). The extinction values are calculated per 100 heads in 1 cc of acid ethyl alcohol. Wave-length 470 mμ. Each value was obtained in a separate experiment.

w^h	w^a	w^{ch}	w^e	w^{co}
0.290	0.490	0.900	0.990	1.970
0.250	0.540	1.020	1.040	2.010
0.280	0.530	1.050	1.010	1.870
0.290	0.530	0.990	0.910	1.880
0.280	0.510	1.090	0.930	2.020
0.260	0.520	1.070	0.860	1.900
0.270	0.530	1.020	0.930	2.000
	0.560	1.100	1.020	2.180
		1.150	0.930	1.820
		1.100	1.020	2.090
				1.980
				2.310
				2.130

[1] Work supported by a grant from the PENROSE FUND of the AMERICAN PHILOSOPHICAL SOCIETY.

the changes in the red pigment component only. Although the effects of the white alleles are the chief object of this paper, pigment changes due to other factors will also be discussed as they aid in the general interpretation.

MATERIAL AND METHODS

The standard technique used for the extraction of red pigment is that described in the first paper of this series (EPHRUSSI and HEROLD 1944). The reduction of pigment content made it necessary, however, to increase the number of heads extracted in approximately inverse proportion to the color of the eyes. Light absorption curves were prepared by use of a Coleman photo-electric spectrophotometer. The data contained in table 1 as well as those used in the preparation of figure 7 were obtained with a Pulfrich photometer.

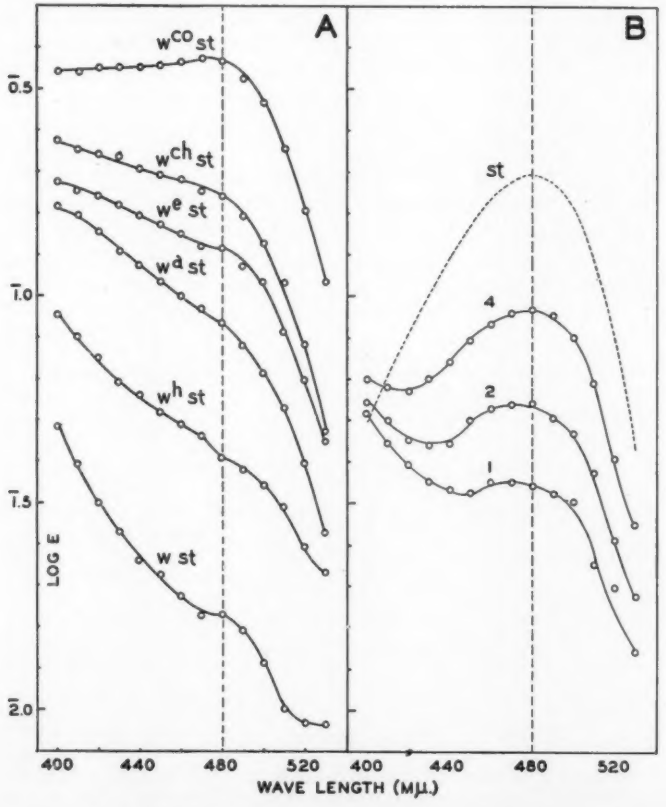

FIG. 1.—A—absorption curves of extracts of red pigment from the mutants (♀ ♀) of the "white" series. B—explanation in text.

The culture conditions were the same as in the paper referred to above. The following mutant alleles were studied: coral (w^{co}); cherry (w^{ch}); eosin (w^{e}); apricot (w^{a}); honey (w^{h}); and white (w). In order to work with flies containing in their eyes only the red pigment all the above mutants were made homozygous for scarlet (st).

LIGHT ABSORPTION BY EXTRACTS OF MUTANT EYES

The absorption curve of the red pigment extracted from the mutant scarlet ($w^{+};st$) has a characteristic bell-like shape with a clear maximum at 480 mμ (fig. 1B, broken line). Extracts of red pigment from the eyes of the mutants w^{co}, w^{ch}, w^{e}, w^{a}, and w^{h} give absorption curves represented in figure 1A. These curves were constructed from data obtained in the same experiment and plotted as *log E*, calculated per 100 heads in one cc of solvent; their shapes are thus independent of concentration. The curves are clearly different from the curve for st and different from each other.

Two features clearly characterize this family of curves: (1) the lower the concentration per eye, the steeper the slope of the curve (that is, the higher the absorption at low wave length relative to that at higher wave length); (2) Although the curves show no characteristic peak in the visible range of the spectrum, they all show a distinct turning point (change of slope) at 480 mμ.

VARIABILITY OF THE RESULTS

The pigment content of eyes of the mutant st varies between rather wide limits depending on the environment (EPHRUSSI and HEROLD 1944). The same

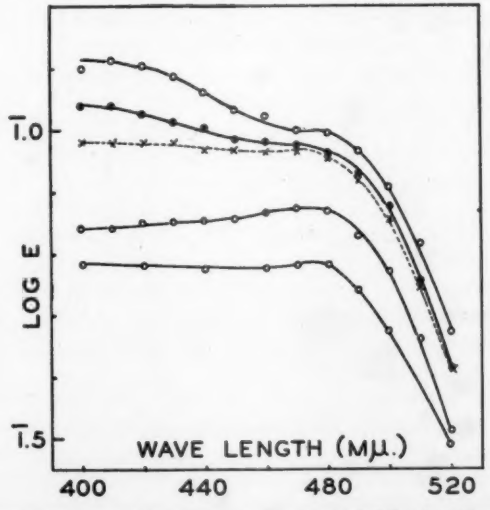

FIG. 2.—Absorption curves of four different w^{co} extracts (full lines). —●—●—●— and --x--x--x--- absorption curves of the same extract before and after oxidation.

may be said of the pigment content of the mutants of the white series and the data of table 1 show the extent of this variation as measured at 470 mμ. This table does not show, however, that the shape of the absorption curves also varies somewhat from experiment to experiment. Figure 2 shows the extremes of variation of the absorption curves of extracts from *w^co^; st* eyes. It can be seen that some of the curves show an almost flat (horizontal) plateau between 400 and 480 mμ., while others have this part of the curve somewhat inclined (sometimes convex), the absorption being highest at 400 mμ. Curves of the former type were obtained in the great majority of experiments. Attempts to find the causes of variation were unsuccessful. It was observed that extracts which at first give sloping curves may give flatter curves after standing for 24 hours and that sometimes the change can be produced instantly by adding a drop of dilute H_2O_2 (compare curves of fig. 2). In the majority of cases, however, the addition of H_2O_2 was not observed to cause any appreciable change in the shapes of the curves of extracts from eyes of the different mutants.

The described variations do not invalidate the comparative characteristics of the absorption curves of the white alleles as revealed by figure 1A. So long as extracts are prepared from flies raised simultaneously and under identical conditions, a family of curves is obtained showing the characteristics described at the end of the preceding section.

ROLE OF IMPURITIES

From the shapes of the absorption curves of figure 1A, which definitely indicate differences between the extracts obtained from different mutants, we may not conclude, however, without further analysis that the pigments contained in these extracts are also different. It is clear that the solvent used (acid ethyl alcohol) is not specific and that the extracts must contain, along with the eye pigments, various impurities. An evaluation of the amount of impurities extracted and of their light absorption can be made by extracting the eyes of the lowest mutant of the series, *w*, which shows no visible coloration. Figure 1A shows an absorption curve given by such an extract. It definitely fits into the family of curves discussed above and this fact suggests that the different curves may be composite and due to *mixtures of a constant amount of impurities with varying amounts of a single pigment*, the purest sample of which is extracted from the mutant *st*.

If this interpretation is correct, one should be able to obtain absorption curves similar to those of figure 1A by adding to an extract of *w* various amounts of a *st* extract. Several experiments of this type were performed, and the results of one of these are given in figure 4.

Five cc of a concentrated extract of *w* heads (a) and a concentrated extract of *st* heads (b) were prepared. Four drops of the latter were first added to 5 cc of pure solvent, and the absorption curve of this solution was determined. The absorption curve of (a) was similarly measured. Thereafter one drop of (b) was added to (a) and the absorption curve of the mixture determined. Further measurements were made after adding 2 and 4 drops of (b). Since the red pigment extracted from *st* follows Beer's law (and we have ascertained that it

does so at all wave lengths here worked with (fig. 3)); and since the extinctions, E, of colored substances are additive when there is no interaction between the latter, the expected extinction of a mixture can be calculated from the extinctions of the mixed components. This has been done, and the results of the cal-

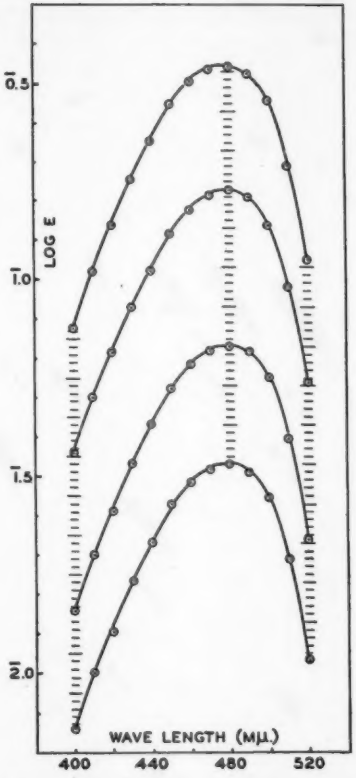

FIG. 3.—Absorption curves of different dilutions of the same *st* extract.

culation are represented by the dotted curves of figure 4, while the full lines give the measured absorption curves. The good agreement between the calculated and observed values shows that there is no interaction between the mixed extracts.[3]

In order to compare the shapes of the curve obtained with those given by extracts of different mutants, the data must first be converted to *log E* values. The curves of figure 1B are the results of such a conversion of the data obtained

[3] The small discrepancies between the expected and experimental values can be accounted for by the dilution of the 5 cc of (a) by the added drops of (b).

in an experiment performed with the same extract of *w* heads which was used in the preparation of the curve of figure 1A (bottom). Figures 1A and 1B are drawn to the same scale so that a quantitative comparison can now be made. Such a comparison shows that the mixture of pigment and "impurities," as

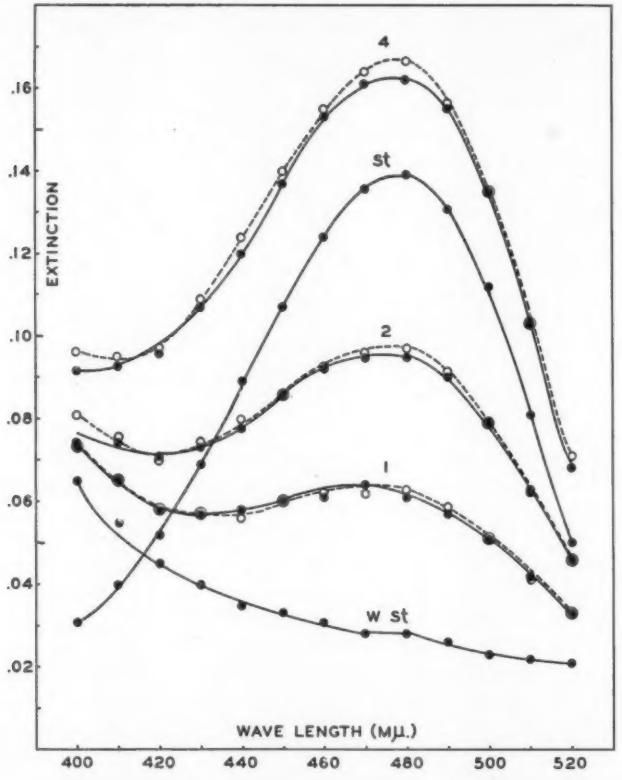

FIG. 4.—Absorption curves of extracts from *w* and *st* eyes, and of their mixtures.

realized in the experiment described, cannot account quantitatively for the shape of the curves given by extracts of the mutant eyes. By using still smaller amounts of (b) mixtures of the two extracts (a and b) could be prepared which would give absorption curves closely resembling some of the curves given by extracts of the higher mutants of the series; but in order to obtain curves of similar shape and with an *absorption as high as that of the mutants*, much higher concentrations of the *w* extract would be required. In other words, the shape and absolute values of the latter curves cannot be explained on the assumption of a constant amount of impurities and varying amount of pigment. The

amount of the so-called "impurities" must increase as we go from lower alleles to higher ones. Thus, in the extracts of the mutants we are dealing either with different pigments or with mixtures in different proportions of at least two colored components.

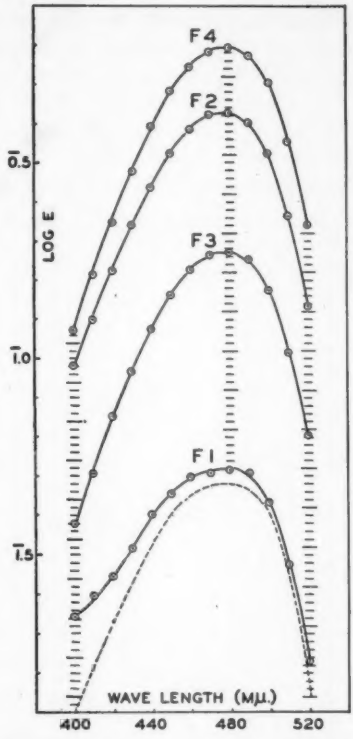

FIG. 5.—Absorption curves of four different fractions of *st* pigment. The dotted curve represents the absorption of F–1 after subtraction of the absorption due to impurities (compare with fig. 3).

FRACTIONATING OF THE SCARLET PIGMENT

The conclusion reached in the preceding section raises first of all the question of homogeneity of the pigment extracted from *st* eyes, which was taken thus far as the standard of comparison. Is this pigment a single chemical entity or is it also a mixture?

It was stated earlier (*l.c.*) that the extraction of red pigment from entire (unsplit) heads is an extremely slow process requiring several days for its completion. This fact has been made use of in an attempt to fractionate the pigment. One hundred *st* flies were decapitated and the heads left on the slide for 15 minutes. This time is sufficient to assure desiccation of the small cut sur-

face. Thereafter the heads were placed in 6 cc of solvent. After a period of extraction of several hours the solution was decanted and replaced by the same amount of fresh solvent. The same procedure was repeated four times. The results of the absorption measurements performed on these four extracts are given in figure 5. The curves show distinct differences in slope, the earlier fractions giving relatively higher absorption at lower wave length.

Are these differences between the absorption curves of the four fractions due to contamination with various amounts of impurities? Assuming that the absorption of the extracts of *w* is entirely due to impurities (which is probably an overestimate as the eyes of *w* may contain some pigment), the extinction due to these impurities may be subtracted from the extinction of fraction F-1 which shows the greatest absorption at low wave lengths. The curve of figure 5

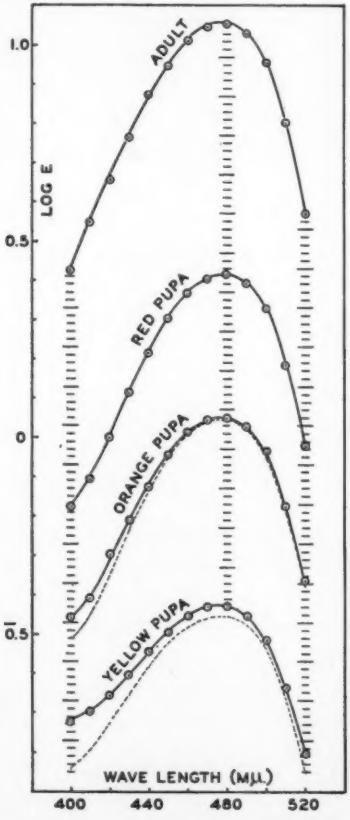

FIG. 6.—Absorption curves of extracts from eyes of *st* pupae of different ages. Dotted curves—absorption after subtraction of absorption due to impurities.

(broken line) is thus obtained, and it can be seen that it still is not parallel to the curves of fractions F-2 and F-3 (which correspond to extractions within 24 hours, that is, the period of extraction of *w*), the remaining difference being in the same direction as before. The differences between the absorption curves must therefore be considered as corresponding to differences between the pigments contained in the four fractions. We thus reach the conclusion that the "red pigment" itself is a mixture of at least two slightly different components.

COLOR CHANGES DURING PUPAL DEVELOPMENT OF SCARLET

The question may now be raised whether the rather striking eye color changes which take place during the development of the flies are due to changes in the proportions of the pigment components revealed by the above observations. A first approach to this question was made by examining the color change,—from yellow through orange to red,—which occurs in the *st* pupa soon after the first appearance of color in the eyes. A great number of pupae were raised, and about 75 hours after puparium formation were separated into three classes according to the eye color: yellow, orange, red.[3] The pupal cases were opened, the flies removed and decapitated, and the extractions performed as on the heads of imagoes.

The results of one of these experiments are given in figure 6 which shows that the shape of the absorption curves changes with the age of the pupae. Even after correction for impurities (by subtraction, as above, of the extinction given

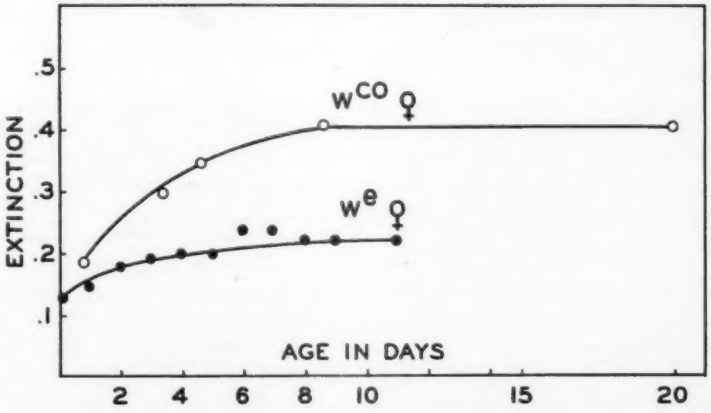

FIG. 7.—Change in content of red pigment with age of w^{co} and w^{e} imagoes (♀ ♀). (Pulfrich, 470 mμ).

[3] The characteristics of the three stages are as follows: "Yellow" pupa: eyes light to dark yellow; wings and bristles colorless to slightly colored; "Orange" pupa: eyes light to dark orange, wings gray to almost fully colored, bristles almost fully colored: "Red" pupa: eyes bright vermilion, wings and bristles black.

per head of *w* flies), the curves remain dissimilar, those corresponding to younger pupae giving relatively higher absorption at the low wave lengths.

Oxidation of the extracts from "yellow pupae" does not change the shape of their absorption curves. Reduction with sodium hydrosulphite causes disappearance of color. Determination of the final pH of the extracts, performed in one of the experiments with the help of a glass electrode, has provided the following values:

	pH
Yellow pupae.....	2.05
Orange "	2.03
Red "	2.03
Pure solvent.....	2.02

Since the described color changes take place during a very short period of time (a few hours), the amount of impurities extracted per head can safely be assumed to remain constant. The conclusion, therefore, appears justified that the eye colors characteristic of the different stages of development of the pupa are due to changes in the composition of the pigment.

"AGE CHANGE" IN CORAL

Considerable darkening of the eye color, known as "age change," occurs during the imaginal life of flies. The age change is particularly marked in the lighter mutants. Figure 7 illustrates the course of the process in w^{co} and w^{e} females and shows that a stable pigment concentration (measured at a single wave length, 470 mμ) is reached only a week or more after hatching of the flies. Is this darkening due to the increase of the concentration of a single pigment?

Great numbers of w^{co} flies were raised and collected within a few hours of emergence. Decapitation and extraction were performed at various times thereafter. The results of one of the experiments are given in figure 8. It can be seen that the increase in the absolute values of extinction with age of the extracted flies is accompanied by a gradual flattening of the absorption curves.

DISCUSSION

The experiments described in the preceding sections have shown that increase of pigment content in the eyes is accompanied by changes of the shapes of the absorption curves of the pigment extracts, whether this increase is due to: (1) substitution of one allele of the *w* series for another: (2) advancing development of the *st* pupa; or (3) "age change" in the w^{co} imago. To these three cases may be added (4) very similar changes in the mutant blood (w^{bl}) described in the second paper of this series (EPHRUSSI and HEROLD 1944, fig. 3) resulting from the action of different temperatures.

What is the cause of these changes in the shape of the absorption curves? The generality, uniformity, gradual character and orderliness of these changes at once suggest that we are dealing with changes in proportion of different components of a mixture, rather than with qualitative differences of single pig-

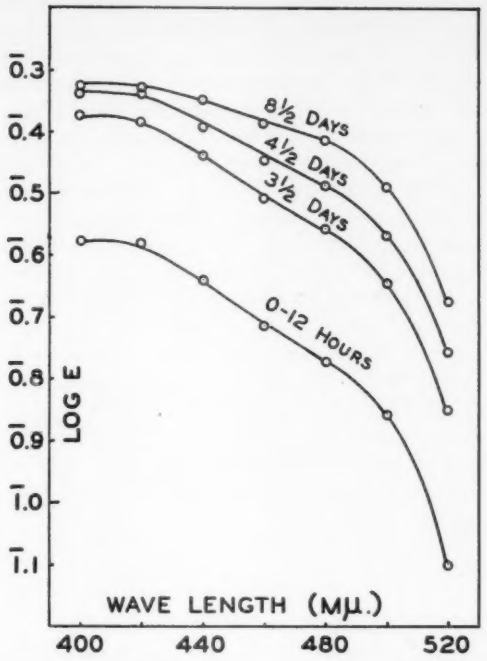

FIG. 8.—Absorption curves of extracts from eyes of w^{co} imagoes of different ages.

ments. This conclusion is further strengthened by the fact that fractionation of the *st* extract seems to permit the separation of slightly different components from what was previously regarded as the purest of the pigments dealt with, thus suggesting its heterogeneity.[4]

It will be recalled on the other hand that in the first of the cases enumerated it has been definitely shown that the changes in the absorption curves cannot be caused by the increasing contamination of the pigment. The same conclusion has been drawn concerning the pigment changes in the second case, and a close similarity of these changes with those observed in cases (3) and (4) makes it unlikely that contamination by impurities is the responsible factor in the latter, although rigorous experimental proof is not available here. Thus the observed changes in the shapes of the absorption curves of the pigment extracts must be considered as due to concomitant variation in at least two colored components.

[4] A qualitative difference due to a different association with a protein carrier is unlikely, since the solvent (acid ethyl alcohol) most probably splits off the chromophore group. It is to be noticed that the difference in the shape of the curves of *st* and w^{co}, described above, is unchanged when 90 per cent acid alcohol is used for the extraction instead of the usual 30 per cent AEA. Extraction of proteins in this solvent is most unlikely.

As pointed out above, mixtures in different proportions of two different pigments (one with an absorption curve similar to that of *w*, the other to that of *st*) could reproduce some curves given by the mutants of the *w* series. But a closer comparison of the curves given by the extracts of mutants of this series with those given by mixtures of *w* and *st* extracts (fig. 1A, B) shows that the curves given by the higher alleles of the *w* series (w^{co} in particular, with its almost horizontal or even somewhat convex plateau) are not likely to be reproduced in the same way. Although the occurrence of unexplained variations in the shape of the absorption curves (of any given mutant) makes this conclusion only tentative, we are inclined to regard it as pointing to the existence, in the different pigment extracts, of more than two colored components.

As to the possible relationship between these different colored components, appearing and disappearing (or, at least, greatly varying in amount) independently of each other under the influence of genetic, environmental and physiological factors, it appears possible to us, as a working hypothesis, to regard them as a continuously varying series of molecular structures, built to varying degrees of complexity from identical building blocks. A process of condensation appears to be one of the possible underlying mechanisms. We thus reach an interpretation somewhat intermediate between a purely quantitative and a purely qualitative one,[5] and we will recall in this connection that a very similar stand has been taken by BECKER (1942) as regards the composition of the brown eye pigments (ommatins) of several insects, on the basis of elementary analysis.

Returning now for a brief review to the four types of changes studied, it seems probable that the different phenotypes of the mutants of the white series represent different levels reached by a single process. In terms of the hypothesis advanced above, these different levels would correspond to different degrees of condensation. The variations observed during pupal development of *st* and in the "age change" in w^{co}, would represent temporal changes in an otherwise similar process. Finally, in the temperature-sensitive mutant w^{bl}, temperature would be the chief parameter setting the limit for the same process.

As applied to the effects of the mutants of the white series, the suggested hypothesis distinctly runs along the lines of GOLDSCHMIDT'S general theory of gene action (GOLDSCHMIDT 1938). A corollary of this theory is the inference, from purely quantitative differences in the gene controlled reactions, to purely quantitative differences between allelic genes. The observations related in the present paper do not impose such a deduction, the general validity of which has been critically discussed by STERN (1930).

SUMMARY

A spectrophotometric study was made of the extracts of red eye pigment of several mutants belonging to the series of multiple alleles at the white locus of

[5] Preliminary experiments point to the existence of considerable differences in the rate of diffusion and the reaction to alkali between the red pigments extracted from w^{co} and *st*. In untreated smears of eyes, the pigment granules appear yellow in the recessive mutants (homozygous for *st*), red in $w^{+}st$.

Drosophila melanogaster. Each of the extracts was found to give a different absorption curve. The higher the allele (that is, the greater the pigment content), the lower the absorption at low wave length (400 mμ.) relative to that at high wave length (480 mμ.). The differences between the different absorption curves are shown to be due to the pigments themselves and not to various degrees of contamination by impurities.

Similar gradual changes in the shape of absorption curves were found to accompany increase of pigment content during pupal development and ageing of the imago.

The nature of these changes is discussed and the hypothesis advanced that they are due to changes in proportion of several colored components corresponding to various degrees of condensation of identical pigment molecules.

LITERATURE CITED

BECKER, E., 1942 Über Eigenschaften, Verbreitung und die genetisch-entwicklungsphysiologische Bedeutung der Pigmente der Ommatin- und Ommingruppe (Ommochrome) bei den Arthropoden. Z.i.A.V. **80**: 157–204.

EPHRUSSI, B., and J. LANE HEROLD, 1944 Studies of eye pigments of Drosophila. I. Methods of extraction and quantitative estimation of the pigment components. Genetics **29**: 148–175. II. Effect of temperature on the red and brown pigments in the mutant blood (w^{bl}).

GOLDSCHMIDT, R., 1938 Physiological Genetics. New York: McGraw-Hill.

MAINX, F., 1938 Analyse der Genwirkung durch Faktorenkombination. Versuche mit den Augenfarbenfaktoren von *Drosophila melanogaster*. Z.i.A.V. **75**: 256–276.

STERN, C., 1930 Multiple Allelie. Handb. Vererbgsw. I, G (Lief. 14), Berlin. Gebr. Borntraeger.

MUTATIONS OF BACTERIAL VIRUSES AFFECTING THEIR HOST RANGE[1]

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INTRODUCTION

WHEN susceptible bacteria are spread on a solid culture medium with a large amount of a bacterial virus, complete lysis occurs after incubation, except for the appearance in some cases of colonies consisting of virus-resistant cells. These are the descendants of bacteria that had undergone a mutation from virus-sensitivity to virus-resistance prior to the action of the virus (LURIA and DELBRÜCK 1943). The virus-resistant bacteria do not adsorb the virus. Their resistance is generally specific, not extending to unrelated viruses. Conversely, when a virus is plated with a suspension of bacteria resistant to its action, it generally does not affect the bacteria; a uniform layer of bacterial growth results. We observed, however, that plating very large amounts of a virus with a resistant bacterial mutant strain occasionally results in the formation of a few clear "plaques"—that is, of a few virus colonies. From these plaques a new virus strain may be isolated that is active on the bacterial mutant resistant to the normal virus. A study of the origin of the new virus proved that it arises by mutation from the normal virus. A mutation of the virus can thus compensate for a mutation of the bacterial host. The present paper is concerned with the study of these virus mutations and of their relation to bacterial mutations.

Mutations affecting characters of bacterial viruses have been described before (GRATIA 1936a; BURNET and LUSH 1936). In 1929, SERTIC clearly recognized the occurrence of true breeding variants of bacterial viruses capable of attacking bacterial strains resistant to the original virus. He appears to have considered such variants as the result of an adaptation of the virus when in the presence of resistant bacteria.

EXPERIMENTAL

Material and basic findings

The material for the experiments described in this paper consisted originally of a strain of *Escherichia coli* **B** and of two viruses, α and γ, active on strain **B** (DELBRÜCK and LURIA 1942). Virus α gives large plaques, virus γ small plaques when plated with **B** on solid media. From strain **B**, a series of mutant strains can be isolated, some sensitive to virus α and resistant to virus γ, others sensitive to virus γ and resistant to virus α. These strains are obtained as secondary growths after lysis of **B** by virus γ or virus α. They are easily purified by repeated streak platings, and their resistance to one of the two viruses is

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generally found to be complete: the virus is not adsorbed by the bacterial cells and does not grow in their presence.

We have adopted in this paper the convention of naming the mutant bacterial strains by the Roman letter corresponding to the original strain, followed by the Greek letter corresponding to the virus in the presence of which they have developed as secondary growth after lysis. For example, strain **B**α indicates a mutant from **B** isolated as secondary growth after **B** was lysed by virus α. This mutant will generally be resistant to virus α (unless otherwise specified). Strains isolated in the presence of the same virus, but different in some properties (colonial morphology, range of sensitivity, etc.), are distinguished by sub-indexes: **B**α_1, **B**α_2, · · · Mutants obtained from other mutants are named by adding the Greek letter corresponding to the virus in the presence of which the new mutant has been isolated: **B**$\alpha\gamma$, for example, will be a mutant from **B**α isolated after lysis of **B**α by virus γ, and will generally be resistant to both viruses α and γ. This notation offers the advantage that the name of each mutant strain mirrors its previous history, indicating the strain of origin and the virus, or viruses, whose selective action has brought about its isolation.

From strain **B**, one can easily isolate two mutants, **B**α_1 and **B**α_2, differing in some growth characteristics but both resistant to virus α and sensitive to virus γ. Very exceptionally, one obtains from **B** a mutant **B**γ, resistant to virus γ and sensitive to virus α.[2]

Plating very large amounts of virus γ with a suspension of cells **B**γ often produces some small, clear plaques, similar to the regular γ-plaques produced on **B**. The ratio of the number of plaques thus produced to the number of plaques produced by the same virus suspension when plated with strain **B** is generally very small (of the order of 10^{-7}, occasionally up to 10^{-5}). For a given suspension of virus, this ratio remains constant, at least for several months. The plaques produced on **B**γ being clear, they cannot be attributed to non-homogeneity of the bacterial suspension, with presence of some γ-sensitive cells: the action of a virus on a bacterial mixture partially sensitive results in the formation of plaques of turbid appearance, due to the growth of the resistant cells.

If some of the contents of one of the few plaques produced by a suspension of virus γ on **B**γ are picked up and immediately plated again with a suspension of **B**γ, a large number of plaques are obtained. This confirms the expectation that the plaque contained a large number of virus particles attacking **B**γ. By means of repeated one-plaque isolations and platings with **B**γ, a pure virus strain is obtained which can be grown in liquid cultures of **B**γ. This pure virus strain we called γ'; its properties will be described in the following section.[3]

It may be stated here that the presence of virus γ' has been found in concen-

[2] The strains **B**γ and **B**α_1 have been called A and C in previous papers (DELBRÜCK and LURIA 1942), in which the interest was focused on their use as indicator strains for plating mixed virus suspensions rather than on their mutational origin.

[3] Several of the experiments with virus γ' were done using a strain **B**$\gamma\alpha_2$ instead of strain **B**γ, because our stock of strain **B**γ (obtained from strain **B** in 1941) had recently, in the course of subculturing, become partially sensitive to virus γ. Strain **B**$\gamma\alpha_2$, derived from **B**γ before this occurred, behaved toward viruses γ and γ' exactly like the original strain **B**γ.

trated suspensions of virus γ prepared directly from one γ-particle (one γ-plaque transferred to a liquid culture of **B**, incubated until lysis, lysate filtered). This proves that virus γ' does not represent an initial non-homogeneity of the stock of virus γ, but arises from normal particles of virus γ.

We have said that at least two different mutants, $B\alpha_1$ and $B\alpha_2$, can be isolated after lysis of **B** by virus α. Plating any amount of virus α with bacteria $B\alpha_1$ does not result in any effect on the bacterial growth. We found, however, that plating very concentrated suspensions of virus α with cells of strain $B\alpha_2$, characterized by slow and limited growth on nutrient agar, often results in the formation of a few clear plaques. These plaques contain a true breeding virus α', active on strains **B** and $B\alpha_2$, but not on $B\alpha_1$. The properties of this virus α' will be discussed in a later section.

The culture media used in this study were: nutrient broth +0.5 per cent NaCl for liquid cultures; the same plus 1.1 per cent powdered agar for platings. All experiments were performed in water-baths or incubators at 37°C. The technique for growth curves of bacteria, growth experiments of the viruses, and experiments on interference between viruses have been described in detail in a previous paper (DELBRÜCK and LURIA 1942). The meaning of certain terms used hereafter, however, may be recalled: *constant period* = minimum time between infection of a bacterium by virus and its lysis with virus liberation; *burst size* = average yield of virus particles per lysed bacterium; *infective center* = anything that produces one plaque when plated with sensitive bacteria. An infective center, therefore, can be either a free particle of virus or a bacterium infected by the virus; both of them will give just one plaque when plated with sensitive bacteria; *efficiency of plating* = the ratio between the number of plaques produced by a virus suspension in a given plating and the maximum number of plaques which that suspension can give when plated under optimum conditions. The latter has been shown to correspond very closely to the actual number of active virus particles present (DELBRÜCK and LURIA 1942).

Properties of virus γ'

Each one of the plaques obtained by plating a suspension of virus γ with $B\gamma$ is capable of yielding a strain of virus γ', which can be purified by repeated platings with $B\gamma$ and one-plaque isolations, since normal virus γ does not grow on bacteria $B\gamma$. After isolation and purification, virus γ' can be grown in liquid cultures of either bacteria **B** or $B\gamma$; filtrates of such cultures yield stable stocks. Whether grown on strain **B** or on strain $B\gamma$, the particles of virus γ' show exactly the same properties, as hereafter described.

The plaques produced by virus γ' on **B** are small, sharp edged, and indistinguishable from those produced by virus γ. Those produced on $B\gamma$ can be distinguished with some experience mainly because of their slightly larger size.

An interesting feature of virus γ' is that of giving a smaller number of plaques when plated with strain $B\gamma$ than with **B**. The efficiency of plating on $B\gamma$ varies from 0.2 to 0.6 of that on **B**. It seemed important to investigate whether or not this difference in the efficiency of plating was due to non-

homogeneity of the particles of virus γ'. It could be imagined that the γ'-particles in reproducing gave origin to a certain proportion of particles of type γ, and therefore active on **B** only. This was excluded by experiments of the following type: Virus γ' was plated with cells **B** in amounts that gave a few hundred plaques. After incubation, the contents of each of 20 plaques were picked up with a needle, inoculated into separate samples of a suspension of cells **B**γ, and immediately plated. All the plates showed numerous plaques, proving that all the plaques produced by the suspension of virus γ' on **B** actually contained virus γ'.

Another conceivable possibility was that a certain proportion of the particles in a suspension of virus γ' produced virus γ' when growing on **B**, but were not themselves capable of attacking cells of type **B**γ. This possibility was excluded by experiments of the following kind, directed to prove that all particles in a suspension of virus γ' can attack **B**γ in liquid media: A definite amount of virus γ', after titration with both bacteria **B** and **B**γ, was added to an excess of growing cells **B**γ. After allowing a few minutes for adsorption (10 minutes, that is, much less than the constant period; see below), the mixture was divided into two portions. One portion was centrifuged for titration of the free virus in the supernatant, the other portion was tested for the number of infective centers, by plating separately with **B** and with **B**γ. Since no liberation of new virus had yet taken place, each infective center represented either a free virus particle or an infected bacterium. If those particles of virus γ' that give plaques on **B** and not on **B**γ are actually unable to attack **B**γ, they will remain free and not appear as infective centers on **B**γ. If, on the contrary, they can be adsorbed by **B**γ, they will account for a part of the infected bacteria. As such, they are likely to appear as infective centers on **B**γ, because each infected bacterium later liberates a large number of virus particles, thereby increasing the chances of plaque formation. This will result in an increase of the number of infective centers relative to the original input of virus as measured by plating with **B**γ. Table 1 shows the results of three such experiments.

It is seen that the number of infective centers on **B**γ after adsorption by **B**γ

TABLE 1

The number of infective centers of virus γ' after adsorption by cells **B**γ.

	EXPERIMENT NO. 13		EXPERIMENT NO. 14		EXPERIMENT NO. 16	
	PLATING WITH **B**	PLATING WITH **B**γ	PLATING WITH **B**	PLATING WITH **B**γ	PLATING WITH **B**	PLATING WITH **B**γ
Virus input per cc	27×10^6	7×10^6	35×10^6	7×10^6	11×10^6	3×10^6
Free virus per cc	8×10^6	2.7×10^6	8×10^6	2.5×10^6	2.7×10^6	1.1×10^6
Infective centers after adsorption per cc	28×10^6	27×10^6	35×10^6	28×10^6	11.5×10^6	10.5×10^6

TABLE 2

Growth of virus γ′ on bacterial strains **B** *and* **Bγ** *in broth at 37°C.*

	STRAIN **B**	STRAIN **Bγ**
Generation time of the bacteria	19 minutes	24 minutes
Virus adsorbed in 5 minutes (7×10^7 bacteria/cc)	80%	less than 10%
Virus adsorbed in 5 minutes (2×10^8 bacteria/cc)	over 90%	40%
Constant period	21 minutes	21 minutes
Burst size	95–130	40–60

is almost as high as the number of infective centers on **B.** The differences are partly accountable for by the free virus, partly by sampling errors. These experiments prove that all particles in a suspension of virus γ′ are capable of attacking cells **Bγ** —that is, they are all γ′-particles.

The low efficiency of plating of virus γ′ with **Bγ** must, therefore, be due to some reason other than those considered above. The most likely explanation is that it is a result of the very low adsorption rate of the virus by these cells, as shown below. Many particles of virus may either remain unadsorbed on the agar or be adsorbed by bacteria too late to produce a fully developed, visible plaque. Any such explanation is bound to be tentative, in view of our incomplete knowledge of the process of plaque formation.

The interaction of virus γ′ with bacteria **B** and **Bγ** was studied by means of adsorption experiments and "one-step growth" experiments (DELBRÜCK 1942). The results are given in table 2. The interaction of virus γ′ with bacteria of strain **B** is in every respect similar to that of virus γ (DELBRÜCK and LURIA 1942). Strain **Bγ** adsorbs virus γ′ much more slowly than strain **B.** In order to obtain measurable adsorption, the experiments with **Bγ** had to be done with older cultures, containing more cells than those used for **B.** The cells in such cultures are likely to be of smaller size, and this makes the values for the adsorption rates and also for the burst size not strictly comparable. An experiment with cells **B** grown to reach the same concentration as used for **Bγ**, however, showed a burst size of 95—that is, not so low as for **Bγ**.

It is interesting to notice that, whereas the bacterial strain **Bγ** has a longer generation time than **B,** virus γ′ grows on **Bγ** with the same constant period as on **B.** The burst size, however, is smaller. The mutation **B**→**Bγ** involves, besides the change in virus sensitivity, changes in the rate of bacterial division and in the yield of virus γ′ per cell.

Interference of virus γ with the growth of virus γ′

Interference between different bacterial viruses growing on the same host has been described and found to conform to the general rule that one cell liberates only virus of one type (DELBRÜCK and LURIA 1942; DELBRÜCK 1944). It was concluded previously that interference also occurs between particles of the same virus strain: when a cell is attacked by several particles of the same strain

(multiple infection), the result is the same as with single infection, as if only one particle could grow in a cell. Direct proof of this "self-interference" was difficult to obtain, since the offspring of a virus particle is indistinguishable from that of another particle of the same virus. Interference with the growth of virus γ by an excess of ultraviolet inactivated virus γ (LURIA and DELBRÜCK 1942) was an indirect confirmation of the occurrence of self-interference.

The availability of virus γ', identical with virus γ in its behavior toward cells of strain **B**, but traceable by its activity on strain **B**γ, offered an opportunity for further study of self-interference. The experiments were done by adding both viruses γ and γ', the former in excess, to cells **B**, then using **B**γ as a plating indicator for virus γ'. If virus γ interferes with the growth of virus γ', a bacterium infected first with virus γ, then with virus γ', will not liberate any virus γ' and will not give a plaque when plated with **B**γ. A loss of infective centers will result.

We give here the data from such an experiment, in which the results were made more clear by the use of anti-virus serum to eliminate the free virus. DELBRÜCK (1944) has found that anti-virus serum inactivates free virus but generally does not affect the growth of virus particles already adsorbed by bacteria.

A culture of **B** was divided into two portions. At time zero one portion received an excess of virus γ, and one minute later a smaller amount of virus γ'. The other portion received only virus γ'. After five minutes, the cultures were diluted into tubes containing a dilution of anti-γ serum sufficient to reduce the amount of free virus (both γ and γ') to about 1 per cent in three minutes (see a later section). At the eighth minute the mixtures were highly diluted into broth to stop the action of serum, and the infective centers of virus γ' were measured by plating with **B**γ at 12 and 17 minutes—that is, before liberation of new virus had taken place. Other platings were done to determine the amounts of free viruses. The results may be summarized as follows:

	I portion	II portion
Bacteria/cc	8×10^7	8×10^7
Absorbed virus γ/cc	80×10^7	——
Adsorbed virus γ'/cc	2×10^7	2×10^7
Infective centers γ'/cc	0.2×10^7	2×10^7

It is seen that in this experiment about 90 per cent of the bacteria infected with both viruses γ and γ' failed to liberate any virus γ'. Other experiments, in which anti-virus serum was not used, gave results of the same type, although the presence of free virus γ' made the loss of infective centers less conspicuous. If we are justified in considering virus γ' as identical to virus γ in its action on strain **B**, we can view these experiments as a direct proof of the occurrence of interference between similar virus particles adsorbed by the same cell.

Properties of virus α'

Virus α', as isolated from the few plaques obtained by plating virus α with **B**α_2, is indistinguishable from virus α in its activity on bacteria of strain **B**. The

plaques are identical, and so is the growth in liquid medium: constant period 13 minutes, burst size 110–130 in broth at 37°C. Plated with bacteria of strain $B\alpha_2$, a suspension of virus α' gives a smaller number of plaques than with **B**. The efficiency of plating is 0.25–0.7. The plaques are of smaller and more variable size. In liquid medium, bacteria $B\alpha_2$ adsorb virus α' extremely slowly, so that reliable adsorption measurements are difficult to obtain. The constant period for growth in broth at 37°C is 13 minutes, similar to the growth on **B**. The burst size is difficult to determine with any degree of accuracy, because the presence of large amounts of unadsorbed virus disturbs the calculation of the number of infected bacteria. From an experiment in which at least a great part of the free virus was eliminated by means of anti-serum, we obtained for the burst size a minimum value of 55.

There are some indications that strains of virus α' independently isolated may differ from one another. The size of the plaques produced on the same bacterial strain is sometimes different. This point has not yet been further investigated.

The mutational origin of viruses α' and γ'

The presence of virus α' in suspensions of virus α and that of virus γ' in suspensions of virus γ are demonstrated by plating a large amount of the suspensions with bacteria resistant to the normal virus. We observe only the end result—that is, the appearance of a few plaques containing a new type of virus. Several alternative modes of origin of the new virus are *a priori* conceivable. Hypothesis 1: There is a small finite probability that, when plated with *resistant* bacteria, a normal virus particle succeeds in attacking one of the resistant cells; when this happens, then the particle will give rise to a virus strain capable of attacking the bacteria resistant to the original virus. Hypothesis 2: There is a small finite probability that a normal virus particle in a culture of virus growing on *sensitive* bacteria mutates, becoming hereditarily capable of attacking the resistant bacteria. Hypothesis 3: There are in a sensitive bacterial culture some exceptional, abnormal bacteria which, when infected by a normal virus particle, produce virus of the new type instead of the normal type.

As far as hypotheses 1 and 2 are concerned, the situation is similar to that encountered in the study of virus-resistant bacteria from virus-sensitive bacterial strains, and analogous considerations apply (LURIA and DELBRÜCK 1943).

According to hypothesis 1, on the one hand, the number of virus particles that succeed in giving plaques on the resistant bacteria should be proportional to the number of virus particles tested. This will be true whether these particles come from the same virus culture or from different cultures, since the particles that produce plaques on the resistant bacteria are normal particles at the time of plating, and the probability of producing a plaque is assumed to be uniform for all particles. If we test a large number of samples each containing the same amount of normal virus, the numbers of plaques produced on the resistant bacteria should show only the fluctuations due to the sampling er-

ror. These numbers should therefore show a Poisson distribution (variance = mean).

According to hypothesis 2, on the other hand, the new type of virus particles stem from mutations occurring during the growth of normal virus on sensitive bacteria, prior to the test. If a mutation occurs before the growth of the virus is completed, the mutant particle will multiply on the normal bacteria and give rise to a clone of mutant particles. The earlier a mutation occurs, the larger the clone will be. If we test a large number of samples, each containing the same amount of normal virus, the result will be different, depending on whether the samples come from the same virus culture or from different virus cultures. If we test different samples of the same culture, we shall again find a Poisson distribution of the number of plaques produced on resistant bacteria. If, however, we test a series of similar virus cultures, all started with a few sensitive bacteria and a few normal virus particles and all containing the same final amount of virus, the numbers of plaques produced will show a distribution with a variance much higher than the mean, because of the presence of clones of mutant particles.

The situation in the case of bacterial viruses is more complicated than in the case of bacteria. The virus particle multiplies by infecting a sensitive bacterium, which, after a latent period, liberates a hundred or more new particles. The mechanism of multiplication of the virus inside the bacterial cell is not known. If the new type of virus arises by mutation during the growth of the normal virus, the distribution of the number of mutant particles will depend on the modalities of the growth of the virus inside the cell.

As far as hypothesis 3 is concerned, if the new type of virus is produced by some abnormal cells, these may be expected to liberate a full burst of particles of the new type. Therefore, we should find that in each virus culture the particles of the new type occur in clones averaging the burst size.

The three hypotheses thus lead to different predictions regarding the distribution of the numbers of plaques produced on resistant bacteria by a series of similar cultures of virus. Accordingly, experiments were undertaken to determine this distribution. In these experiments, a small number of bacteria of strain **B** (about 10^3/cc) were added to a broth suspension of either virus α or virus γ containing 10^3–10^4 particles/cc. The mixture was immediately divided into portions of 0.2 or 0.5 cc and these were incubated at 37°C. Upon incubation, the cultures of virus γ always remained clear (complete lysis, due to the rarity of the mutation $\mathbf{B} \rightarrow \mathbf{B}\gamma$). Cultures of virus α generally also gave complete lysis; only occasionally a few cultures prepared under such conditions showed secondary growth of resistant bacteria. This is explained by the fact that generally in such cultures complete lysis takes place before the bacteria reach a titer high enough to render the occurrence of mutations $\mathbf{B} \rightarrow \mathbf{B}\alpha$ likely. If exceptionally a mutation $\mathbf{B} \rightarrow \mathbf{B}\alpha_1$ occurs, the bacteria $\mathbf{B}\alpha_1$, resistant to both viruses α and α', grow to saturation. If a mutation $\mathbf{B} \rightarrow \mathbf{B}\alpha_2$ occurs, the mutant cells grow to saturation if no α'-particle is present. If α'-particles are present, they are adsorbed by the cells $\mathbf{B}\alpha_2$ when the concentration of the latter is high enough, and the result is a culture containing a large amount of virus α', com-

TABLE 3

Distribution of the numbers of plaques produced on $B\alpha_2$ *by a series of similar cultures of virus* α.

EXPERIMENT NO.	112	113	114	115a	115b
Number of cultures tested	20	34	87	11	40
Volume of each culture, cc	.2	.5	.5	.5	.5
Virus α per culture	1.2×10^8	7×10^8	8.9×10^8	9×10^8	7.8×10^8
NUMBER OF PLAQUES PRODUCED ON $B\alpha_2$	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES
0	16	9	3	2	6
1	1	5	2	2	1
2	3	6	1	2	2
3	0	2	2	1	4
4	0	1	2	0	1
5	0	1	4	0	1
6	0	1	3	0	1
7	0	0	2	0	2
8	0	1	1	0	1
9	0	1	2	0	0
10	0	0	1	0	0
11–20	0	3	11	1	9
21–50	0	1	14	2	10
51–100	0	1	13	1	1
101–1000	0	1	21	0	1
over 1000	0	1	5	0	0
Average per culture	.35	45	125	13.7	15.6
Variance	.57	1200	45,000	570	380

parable to its titer of virus α. In our experiments all these possibilities were actually realized: we found several cultures with growth of $B\alpha_1$, one culture with growth of $B\alpha_2$, and one culture completely lysed but containing such a large amount of virus α' as to suggest that virus α' had multiplied on a secondary growth of $B\alpha_2$.

After incubation, the completely lysed cultures were tested for the amount of virus α (or virus γ) and for the number of plaques produced on α-resistant (or γ-resistant) bacteria. For the latter purpose, the whole contents of each culture were plated according to the technique devised by GRATIA (1936b) and by HERSHEY et al. (1943). The results are given in tables 3 and 4. The amounts of virus α (or virus γ) represent averages; the individual counts of normal viruses in different cultures within each experiment never vary more than can be accounted for by the sampling errors. In considering the counts from the platings with the resistant bacteria, we must remember that for both virus α' and virus γ' the efficiency of plating is low and slightly variable from one experiment to another. The plaque counts should be too low by a factor corresponding to the efficiency of plating. It is worth recalling that this factor, if constant within one experiment, should not affect the type of distribution *if*

TABLE 4

Distribution of the numbers of plaques produced on B_γ *by a series of similar cultures of virus* γ.

EXPERIMENT NO.	27	28a	28b	28c	28d	29
Number of cultures tested	20	9	9	9	9	40
Volume of each culture, cc	.2	.2	.2	.2	.2	.2
Virus γ per culture	20×10^8	16×10^8	15×10^8	22×10^7	6×10^7	15×10^7
NUMBER OF PLAQUES PRODUCED ON B_γ	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES
0	1	2	3	6	8	25
1	0	0	0	1	0	8
2	0	2	4	2	0	2
3	1	1	1	0	0	3
4	2	2	0	0	0	2
5	1	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	2	0	0	0	0	0
9	1	0	0	0	0	0
10	0	0	0	0	0	0
11–20	4	2	0	0	0	0
21–50	3	0	0	0	0	0
51–100	2	0	0	0	1	0
101–1000	2	0	1	0	0	0
over 1000	1	0	0	0	0	0
Average per culture	190	4.8	55	.55	8	.72
Variance	215,000	24	27,000	.82	540	1.4

this is a Poisson one. The experimental distribution will be derived from the theoretical one by multiplying the mean by the efficiency of plating and will still be a Poisson distribution.

It is immediately seen in tables 3 and 4 that the fluctuations of the numbers of plaques produced on the resistant bacteria are much higher than could be accounted for by the sampling errors. The variance is generally much higher than the mean, in accord with the expectation from the hypothesis that the new virus arises by mutation from the normal virus.

Another result evident in tables 3 and 4 is the presence of a large proportion of low values, well below the value of the burst size. This suggests that a bacterium may liberate a mixture of normal and mutant viruses and seems to exclude the possibility that the new virus be produced in full bursts by some abnormal bacteria, according to hypothesis 3. The new virus particles arise by mutation in the course of the multiplication of normal virus inside the cell.

It would now be desirable to compare the experimental distribution of the number of mutant virus particles with the distributions to be expected accord-

ing to various conceivable mechanisms of multiplication of the virus inside the bacterial cell, thus proving or disproving the correctness of each of these mechanisms. This comparison, however, is hampered by the low efficiency of plating of both the mutant viruses α' and γ'. Some of the theoretical distributions are not completely known, and the effect on them of the low efficiency of plating cannot be predicted. This is true, for instance, of the distribution encountered in the study of bacterial mutations (LURIA and DELBRÜCK 1943), which would apply with some modifications to our case if the virus multiplied in the cell as bacteria multiply in a culture. Besides, the efficiency of plating may not be exactly constant even within each experiment, and its variations may affect any distribution. Further discussion of this point is postponed until experimental data obtained under conditions in which the efficiency of plating is equal to one will be available. Experiments in this direction are now in progress.

Similar considerations make it pointless to attempt figuring values for the rates of mutation from the data in tables 3 and 4. Obviously, the correct mode of calculating mutation rates depends on the unknown mechanism of virus multiplication.

Antigenic relation of normal and mutant viruses

Bacterial viruses injected into the animal body stimulate the production of antibodies, of which the best known property is their specific virus-neutralizing power. Antisera against viruses α, γ, and γ' were prepared by repeated injections into white rabbits, until the homologous titer had reached a satisfactory level. Cross-inactivation tests were then performed. It was found that, within the experimental precision, a serum neutralized virus α and virus α', or virus γ and virus γ', with the same titer and at the same rate. As an example, we give the values of the fractional rate of neutralization (HERSHEY et al. 1943) obtained by testing the same dilutions of various sera on normal and mutant viruses:

Serum anti-γ : $k_\gamma = 1.3$; $k_{\gamma'} = 1.0$.
Serum anti-γ': $k_\gamma = 0.8$; $k_{\gamma'} = 0.8$ (approximate values).
Serum anti-α : $k_\alpha = 0.3$; $k_{\alpha'} = 0.35$.

No cross-inactivation was found between viruses α and α' on the one hand and viruses γ and γ' on the other hand. For virus γ', it was found that both its activities on **B** and on **B**γ were neutralized at the same rate.

These results clearly show that, whatever structural differences exist between a virus and a mutant derived from it, they are not revealed by serological tests, at least of this rather simple nature.

The virus mutations in relation to the bacterial mutations

Arising by mutation from viruses α and γ, viruses α' and γ' acquire the ability to attack bacterial strains that by mutation have lost their sensitivity to the original virus. The virus mutations compensate for the bacterial mutations. The mutation $\gamma \rightarrow \gamma'$ is complementary to the mutation **B**$\rightarrow$**B**γ; the mutation $\alpha \rightarrow \alpha'$ is complementary to the mutation **B**$\rightarrow$**B**α_2. That the compensation

is not complete is shown by the fact that the mutant viruses are adsorbed by the mutant bacteria more slowly than by the normal bacteria. One may say that the affinity of the mutant viruses for the mutant bacterial strains is poorer than for strain **B**.

Strain **B**γ is sensitive to virus α and to virus γ'. Does the mutation $\gamma \rightarrow \gamma'$ involve the acquisition by the virus of the same structural configuration that enables virus α to attack strain **B**γ? If so, mutants from strain **B**γ which are resistant to virus α should also be resistant to virus γ'. It was easy to isolate from **B**γ several α-resistant mutants, called **B**$\gamma\alpha$; all of them proved perfectly sensitive to virus γ' (fig. 1). Sensitivity to viruses α and γ' cannot, therefore, be due to the same structural configuration.

It was more difficult to isolate bacterial mutants stably resistant to virus γ'. Eventually, we obtained from strain **B**α_1 a mutant strain **B**$\alpha_1\gamma'$ that proved resistant to virus γ and to virus γ', and, surprisingly enough, sensitive to virus α. The sensitivity of strain **B**$\alpha_1\gamma'$ to virus α was unexpected, since this strain was a mutant from strain **B**α_1, which was completely resistant to virus α. The mutation **B**$\alpha_1 \rightarrow$**B**$\alpha_1\gamma'$, therefore, involved loss of sensitivity to viruses γ and γ', but gain of sensitivity to virus α.

It may be added that from strain **B**$\alpha_1\gamma'$ a mutant **B**$\alpha_1\gamma'\alpha$ can easily be obtained, resistant to all three viruses α, γ, and γ' (fig. 1).

Let us now consider the situation concerning virus α'. The mutation $\alpha \rightarrow \alpha'$ involves gain of ability to attack the α-resistant mutant **B**α_2. This ability is not due to the same configuration which enables virus γ to attack **B**α_2, as proved by the fact that virus α' also attacks a mutant strain **B**$\gamma\alpha_2$, resistant to virus γ. Differences in the configurations of virus α' and virus γ' are brought out by their relations to the mutants **B**$\gamma\alpha_1$ and **B**$\alpha_1\gamma'$ (fig. 1). The remarkable point here is that the mutation $\alpha \rightarrow \alpha'$ compensates for only certain mutations of α-sensitive bacteria—namely, **B**$\rightarrow$**B**α_2 and **B**$\gamma \rightarrow$**B**$\gamma\alpha_2$, but not for the mutations **B**$\rightarrow$**B**α_1 or **B**$\gamma \rightarrow$**B**$\gamma\alpha_1$. It is worth mentioning that both strains **B**α_2 and **B**$\gamma\alpha_2$ belong to the group of "small colony" mutants that grow poorly and slowly on nutrient agar, whereas **B**α_1 and **B**$\gamma\alpha_1$ are normal colony formers. This is an example of a modification of the cultural, physiological properties of the bacterial cells correlated with the mutational change to virus-resistance.

The results summarized in figure 1 indicate that various bacterial mutations leading to resistance to unrelated viruses are generally independent of each other. For instance, the same two mutations (sub-1 and sub-2) leading to resistance to virus α (with or without resistance to virus α' and change in cultural characters) can occur either in strain **B** or in strain **B**γ, in spite of the fact that the latter has already undergone a mutation toward resistance to virus γ.

Figure 1 also shows the occurrence of α'-resistant strains, all of which are also resistant to virus α.

The results of this section show that some, if not all, mutational changes in virus sensitivity by our bacterial strains can be compensated for by complementary mutations of the viruses. It is possible that in cases of bacterial mutations for which complementary virus mutations have not been found (for ex-

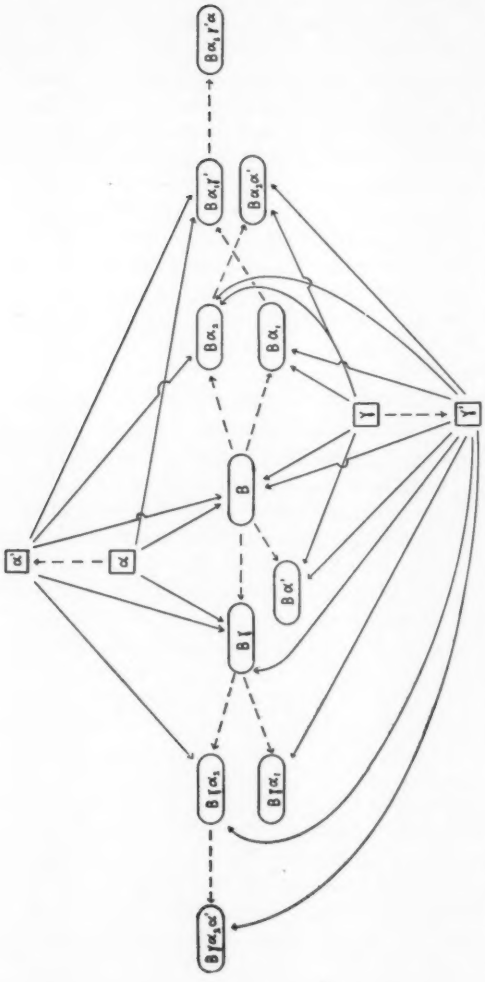

FIG. 1.—The sensitivity relations between mutant bacterial strains and virus mutants. Broken arrows indicate mutations. Solid arrows indicate activity of a virus on a bacterial strain.

ample, $B \rightarrow B\alpha_1$), the virus mutations occur with a frequency too low to make them detectable in virus suspensions of the usual titers.

It is clear from the results obtained with viruses α and α' that loss of sensitivity to a given virus can be brought about, in the same bacterial strain, by different mutations, leading to differences in sensitivity to another virus closely related to the first.

DISCUSSION

The results described above demonstrate that, whereas bacteria can be altered by mutation in their susceptibility to bacterial viruses, the latter can in turn acquire by mutation the ability to attack new bacterial strains. The reverse change, by which a virus particle would lose by mutation the capacity of attacking a certain bacterium, might conceivably occur, but would be difficult to demonstrate, except in cases where it occurred very frequently.

The changes in virus properties are here called "mutations" because of their apparently spontaneous and random occurrence, of their transmission to the offspring, and of their stability. The same may be said of the bacterial mutations affecting virus-sensitivity. In making any analogy with the process of gene mutation in plants and animals, we should not forget the lack of any direct evidence of the presence, in bacteria or viruses, of "genes" in the sense of discrete material units, whose existence in higher organisms is proved by linkage studies.

As for the structural changes involved in the virus mutations, we have seen that the serological tests failed to reveal any difference between original and mutant strains. For the time being, our only basis for attempting to understand the structural changes involved in the mutations is the infectivity of the viruses for different bacterial strains.

The virus-host interaction involves as first step a process of specific adsorption. The specificity of the adsorption of a virus by a bacterium is generally conceived as due to the presence of "receptors" for the virus on the bacterial surface (see BURNET 1930). Adsorption is conditioned by complementarity of the surface structures of the receptor and of the virus, which enables them to fit together. Since the mutant bacteria resistant to a virus do not adsorb that virus, we may assume that the bacterial mutation causes a change in the receptors. It has long been known (BURNET 1930) that changes in virus-sensitivity of bacterial strains are often accompanied by changes in the antigenic make-up of their surface, and some of the antigens have been supposed to be responsible for virus adsorption.

The fact that a change in the surface structure of a bacterium can be compensated for by an independent change in the virus particle suggests that the changes involved are relatively small, possibly limited to simple stereochemical rearrangements, suppressing or restoring the complementarity of the surface structures. The fact that normal and mutant viruses are serologically indistinguishable speaks in favor of this conception. It is interesting to recall that STANLEY (1943) and his collaborators found that strains of tobacco mosaic virus, supposed to be closely related to each other, showed serological relation-

ship, common structural pattern in X-ray diffraction studies, and similarity of gross chemical composition, but showed differences in finer chemical composition.

The low rate of adsorption of the mutant bacterial viruses by their new hosts may be attributed to a less satisfactory fitting of the surface structures of virus and bacterium, lowering their affinity.

A possible alternative explanation must be mentioned—namely, that the acquired ability of a mutant virus to be adsorbed by a new host may be due to a change in only one out of a number of surface structures of the virus particle. This would explain the smaller adsorption rate of the mutant viruses by the mutant bacteria, while the rate of adsorption by the normal bacteria remains the same as for normal viruses.

The evolutionary implications that the results discussed above present for the system bacterium-virus are of some interest. Because of the lytic activity of most bacterial viruses, a bacterial strain is doomed to destruction once it has come in contact with a virus active upon it. The only chance of survival of the bacterial strain is the occurrence of mutations to virus-resistance. As we have seen, however, this occurrence does not necessarily protect the strain, because its mutation to virus-resistance may be compensated for by an independent complementary mutation of the virus. In certain cases (SERTIC 1929) parasitism may be maintained by two parallel series of complementary mutations in the host and the parasite.

Mutations of bacterial viruses enlarging their host range need not always be limited to activity upon closely related bacterial strains, but may conceivably render a virus active on strains belonging to different species. It is interesting, from the standpoint of bacterial taxonomy, that while bacterial viruses may be active on species belonging to different genera, chiefly within the family Enterobacteriaceae, no virus has ever been found to be active on members of different families.

SUMMARY

From two bacterial viruses, α and γ, two new viruses α' and γ' were isolated, differing from α and γ by their ability to attack bacterial strains which by mutation had become resistant to virus α or to virus γ.

An analysis of the distribution of the particles of a new virus in a series of similar cultures of normal virus proved that the new virus arises by mutation from the particles of normal virus in the course of their growth on sensitive bacteria.

Each mutant is indistinguishable from its parent virus in serological properties and in its activity on the common bacterial host. The latter property was utilized to study the interference between similar virus particles. The results confirmed the conclusion that only one of the infecting particles succeeds in growing in each bacterial cell.

The mutant viruses are poorly adsorbed by their new hosts. Their growth on these was investigated.

The sensitivity of a series of bacterial mutants to viruses α, α', γ, and γ' was studied. It was found that resistance to virus α may be brought about in the same bacterial strain by different mutations. These lead to differences in sensitivity to the related virus α' and in other physiological properties.

Bacterial mutations leading to resistance to a mutant virus lead also to resistance to the parent virus.

Bacterial mutations leading to resistance to unrelated viruses generally prove independent; the same mutation can occur in strains with a different history of previous mutations. One exception to the independence of various mutations was found. A bacterial strain resistant to viruses α and α' reverted to sensitivity as a consequence of a mutation to resistance to viruses γ and γ'.

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LITERATURE CITED

BURNET, F. M., 1930 Bacteriophage activity and the antigenic structure of bacteria. J. Path. Bact. **33**: 647–664.

BURNET, F. M., and D. LUSH, 1936 Induced lysogenicity and mutation of bacteriophage within lysogenic bacteria. Aust. J. Exp. Biol. Med. Sci. **14**: 27–38.

DELBRÜCK, M., 1942 Bacterial viruses (Bacteriophages). Advances in Enzymology **2**: 1–32.

1944 Unpublished experiments.

DELBRÜCK, M., and S. E. LURIA, 1942 Interference between bacterial viruses. I. Arch. Bioch. **1**: 111–141.

GRATIA, A., 1936a Mutation d'un bactériophage du *Bacillus megatherium*. C. R. Soc. Biol. **123**: 1253–1255.

1936b Des relations numériques entre bactéries lysogènes et particules de bactériophage. Ann. Inst. Pasteur **57**: 652–676.

HERSHEY, A. D., G. KALMANSON, and J. BRONFENBRENNER, 1943 Quantitative methods in the study of the phage-antiphage reaction. J. Immunol. **46**: 267–279.

LURIA, S. E., and M. DELBRÜCK, 1942 Interference between bacterial viruses. II. Arch. Bioch. **1**: 207–218.

1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**: 491–511.

SERTIC, V., 1929 Procédé d'obtention de variantes du bactériophage adaptées à lyser des formes bactériennes secondaires. C. R. Soc. Biol. **100**: 612–614.

STANLEY, W. M., 1943 Chemical structure and the mutation of viruses. In: Virus diseases. Ithaca: Cornell University Press.

ABNORMAL SEGREGATION DURING MEGASPOROGENESIS IN MAIZE

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THE principle of random segregation of contrasting alleles during megasporogenesis and microsporogenesis is commonly accepted. Knobs on the chromosomes of maize may be considered as alleles which are visible under the microscope. Any deviation from a random orientation and distribution of bivalent chromosomes during meioses that lead to aberrant genetic ratios and nonrandom segregation of knobs is considered abnormal.

Observations made during the study of chromosome knobs of maize suggested that the segregation of paired chromosomes, one with and the other without a knob, was not strictly random. Recently RHOADES (1942) discovered a preferential segregation at megasporogenesis of plants heterozygous for the abnormal and normal types of chromosome 10 in which approximately 70 per cent of the basal cells of the linear tetrad received the abnormal type of this chromosome. His studies suggested that a more critical study be made of the accumulating data on knob segregation and the segregation of some contrasting alleles known to be near the knob locus of different chromosomes.

Acetocarmine smear preparations of pollen mother cells were used to determine the number and position of knobs in the parent strains, in random samples of all F_1 progenies, and in a limited number of progeny plants obtained from the backcrosses. The number and position of the knobs of parent strains were checked each season as sib-pollinated and self-pollinated seed replaced the original stocks. Determinations of the knobs present in F_1 plants served only as a check on the knob conditions of the two parents that had been combined.

Genetic studies of preferential segregation of contrasting genes were possible through the use of several seed and seedling characters obtained from the MAIZE GENETICS COOPERATION, DEPARTMENT OF PLANT BREEDING, CORNELL UNIVERSITY, Ithaca, New York. These were crossed with the contrasting dominant alleles carried in chromosomes possessing the contrasting knobbed or knobless condition. Two of these studies on the segregation of genes on chromosome 10, three on chromosome 9, and one on chromosome 6 are included in this report.

Since it was convenient in preparation of the tables to have some symbol for the knobbed and knobless condition of a chromosome, the symbols K for knobbed, k for knobless, and $K(S)$ for a small knob have been used. The values of χ^2 have been computed for each population and are reported in the tables. Since each distribution has only one degree of freedom, a χ^2 value of 3.8 indi-

cates odds of 19:1 and a value of 6.6 indicates odds of 99:1 against the deviations being due to chance alone.

EXPERIMENTAL RESULTS

Cytological studies

Each of the ten maize chromosomes may exhibit one or more knobs at the prophase of the first reduction division. Many strains, however, have few or even no knobs on their chromosomes. Consequently, selected strains may be crossed to produce F_1 plants with many of the homologous pairs of chromosomes heterozygous for knobs. When such F_1 individuals are backcrossed to a strain without knobs, the number of knobs found on the chromosomes of the resulting plants will be the number present in the gametes of the heterozygous parent. Any deviation of the mean number of knobs carried by the backcross population from the mean of the two original parents will indicate that there has been a nonrandom segregation in the F_1 plants at megasporogenesis.

Some of the knobbed parent maize strains used in producing F_1 progenies carried the abnormal type of chromosome 10 used by RHOADES (1942) in his studies of preferential segregation. Data on its segregation are included with

TABLE 1

Knob segregation at megasporogenesis of maize.

CHROMOSOME NO.	CHROMOSOME ARM	TOTAL NUMBER OF CHROMOSOMES	KNOBBED CHROMOSOMES		χ^2
			NUMBER	PERCENTAGE	
1	Short	136	79	58.1	3.56
	Long	108	68	63.0	7.26
2	Short	59	35	59.3	2.05
	Long	93	55	59.1	3.11
3	Short	113	72	63.7	8.50
	Long	169	116	68.6	23.49
4	Long	106	87	82.1	43.62
5	Long	77	56	72.7	15.91
6	Long	212	125	59.0	6.81
	Long	212	138	65.1	19.32
8	Long	90	57	63.3	6.40
9	Long	207	148	71.5	38.27
Total		1,582	1,036	65.5	Highly sig.
10	Long	142	106	74.6	34.51

TABLE 2

Summary of knob segregation in megasporogenesis and microsporogenesis of maize.

CHARACTER OF SEGREGATION	TOTAL CHROMOSOMES	KNOBBED CHROMOSOMES		χ^2
	NUMBER	NUMBER	PERCENTAGE	
Plants segregating for Abnormal 10				
Megaspores with Abnormal 10	918	616	67.10	107.4
Megaspores with Normal 10	664	420	63.25	46.65
Microspores (both types of 10)	115	62	53.91	.70
Plants with Normal 10 only				
Megaspores	195	122	62.56	12.31

the cytological data on the segregation of the knobs on the other nine chromosomes.

Data on knob frequency from counts made on plants of the first backcross generation are shown in tables 1 and 2. These data are not all from the same cross and so all knobs are not equally represented. The data on the segregation of individual knobs at megasporogenesis are given in table 1. Some data were obtained on the segregation of 12 different knobs. Each knob was present in well above the expected 50 per cent of the plants of the first backcross generation, thus giving definite evidence of preferential segregation of the knobbed over the knobless homologues at megasporogenesis of the F_1 plants. All these F_1 plants were also segregating for the abnormal and the normal types of chromosome 10. The preferential segregation of 74.6 per cent for the abnormal type substantiates RHOADES' (1942) data and approximates the figures he gives.

The data in table 1 are from two kinds of plants, those from eggs with and those from eggs without the abnormal chromosome 10. A summary of the prevalence of knobs in these two kinds is given in lines 1 and 2 of table 2. The data show that the megaspores of F_1 plants that receive the abnormal chromosome 10 receive 67.1 per cent of the other knobs segregating, whereas the megaspores receiving the normal chromosome 10 receive 63.25 per cent of the knobs segregating. These figures indicate that preferential segregation of knobs at megasporogenesis is the rule and is slightly more biased when the abnormal type goes to the basal cell of the linear tetrad. The data on the same 12 knobs in the last line of table 2 substantiate those in line 2 and indicate that when knobbed and knobless chromosomes segregate, the former are included in the megaspore more than the expected 50 per cent even when the abnormal chromosome 10 is not present.

The meager data in line 3, table 2, show only a slight departure from the expected 50:50 distribution of knobs in the functioning pollen of F_1 plants in which there was an opportunity for the segregation of paired knobbed and knobless chromosomes and of the segregation of the two types of chromosome

10. These data from heterozygous F_1 plants do not distinguish between the possibility of abnormal segregation at microsporogenesis, which might give two of the cells of the tetrad many knobs and the other two but few, and the possibility of a differential viability between pollen with few and pollen with many knobs. However, the number of knobs present in the plants of the first backcross generation indicates that the presence of knobs does not handicap pollen in effecting fertilization.

Genetic studies

When this study was extended to include the segregation of contrasting genes at loci close to knob loci, it was apparent that morphological studies of the chromosomes could not be made of all plants. A knowledge of the knobs present in the parent strains is essential. A check, however, on the homozygosity of the parent strains was kept by determining the number and position of knobs in a random sample of all F_1 progenies. Determining the effect of preferential segregation of the knobbed over the knobless type of a chromosome on the segregation of closely linked genes required no additional cytological determinations. If the paired homologues carrying the contrasting segregating alleles were at a locus so close to the knob locus that little or no crossing-over was taking place, the ratio of recovery of the two contrasting alleles should measure as accurately as cytological determinations the amount of preferential segregation.

The ratio of contrasting genes recovered in backcrossed and selfed progenies expected from random segregation at megasporogenesis and microsporogenesis is 50:50 in progenies from backcrossed plants and 75:25 in progenies from selfed F_1 plants. A deviation from these ratios was expected in the segregation of contrasting alleles whenever the preferential segregation shown by the cytological data was affecting the same chromosomes. The amount of departure of a ratio from that for random segregation also should be a measure of the distance between the knob locus and the locus of the segregating genes if there is no differential viability.

Segregation of the *R r* and *G g* genes

It has been shown that more than 70 per cent of the megaspores of plants in which the two types of chromosome 10 are present receive the abnormal type. Tables 3 and 4 show that this preferential segregation affects the segregation of the contrasting alleles *R* and *r* and *G* and *g*, the former at a locus close to and the latter at a locus an appreciable distance from the end of the long arm of chromosome 10. The data on the segregation of these two pairs of contrasting alleles at megasporogenesis approximate those given by RHOADES (1942) for their preferential segregation and serve merely to substantiate his studies.

The data in line 3, table 3, are from the reciprocal cross of those shown in line 1 and shows no appreciable deviation from the 50 per cent expected from random segregation at microsporogenesis and equal viability of the two types of pollen. Similar conclusions are drawn from the data in lines 2 and 4 of table 4 which show the segregation of the contrasting *G g* alleles. The 81.93 per cent of

TABLE 3

Segregation of the dominant and recessive R r alleles.

PARENTS		TOTAL SEEDS NUMBER	COLORLESS SEEDS		χ^2
♀	♂		NUMBER	PERCENTAGE	
r Ab. 10 / *R* Nor. 10	*r* Nor. 10	35,110	24,229	69.01	Highly sig.
r Nor. 10 / *R* Nor. 10	*r* Nor. 10	5,923	2,892	48.83	3.26
r Nor. 10	*r* Ab. 10 / *R* Nor. 10	226	110	48.67	.16

green plants recovered (line 2) from selfed progenies fits closely the calculated expected 81.52 per cent when the 63.04:36.96 ratio (table 4, line 1) of the two types of eggs is combined with an assumed 50:50 ratio of the two types of pollen, and the 73.96 per cent of green plants recovered (line 4) is close to the 75 per cent expected from random segregation of the contrasting alleles.

Segregation of the paired *C c* genes

The data from several tests of segregation of the *C c* pair of genes for aleurone color are given in table 5. There is approximately 23 per cent recombination between *C* and the end of the short arm. Data on the segregation at megasporogenesis of chromosome 9 in plants heterozygous for the two types of chromosome 10 are shown in the first line. The 63.87 per cent of colored seeds recovered is a definite departure from the expected 50 per cent and shows a preferential segregation at megasporogenesis of the chromosome 9 bearing a knob and the dominant allele *C*. The next two lines of table 5 serve as checks to the data in line 1 and show approximately random segregation of the dominant *C* allele at megasporogenesis both when it is on a knobbed and when it is on a

TABLE 4

Segregation of the dominant and recessive G g alleles.

PARENTS		TOTAL SEEDS NUMBER	GREEN SEEDLINGS		χ^2
♀	♂		NUMBER	PERCENTAGE	
G Ab. 10 / *g* Nor. 10	*g* Nor. 10	1,196	754	63.04	8.14
G Ab. 10 / *g* Nor. 10	Self	20,008	16,393	81.93	Highly sig.
G Nor. 10 / *g* Nor. 10	*g* Nor. 10	1,042	550	52.78	3.23
G Nor. 10 / *g* Nor. 10	*G* Ab. 10 / *g* Nor. 10	2,857	2,113	73.96	1.65

knobless type of chromosome 9 and paired with the recessive *c* allele on a knobless type of 9. Since the abnormal type of chromosome 10 is absent in these two tests, it may be concluded that the presence of the abnormal type of chromosome 10 is associated with the preferential segregation of the knobbed type of chromosome 9. This preferential segregation of chromosome 9 is confirmed by the data in line 5 of table 5 in which 81.28 per cent colored seeds were obtained. This is a very good fit to the expected 82.27 per cent calculated by combining 63.87 per cent *C* bearing eggs with a 50.92 per cent (line 4, table 5)

TABLE 5

Segregation of the dominant and recessive C c alleles.

PARENTS ♀		PARENTS ♂		TOTAL SEEDS NUMBER	COLORED SEEDS NUMBER	PERCENTAGES OF COLORED SEEDS OBSERVED	PERCENTAGES OF COLORED SEEDS EXPECTED WITH RANDOM SEGR.	χ^2
$\frac{C\ K}{c\ k}$	Ab. 10 / Nor. 10	*c k*	Nor. 10	43,523	27,799	63.87	50	Highly sig.
$\frac{C\ K}{c\ k}$	Nor. 10	*c k*	Nor. 10	29,995	14,878	49.60	50	1.90
$\frac{C\ k}{c\ k}$	Nor. 10	*c k*	Nor. 10	12,415	6,106	49.15	50	3.32
c k	Nor. 10	$\frac{C\ K}{c\ k}$	Ab. 10 / Nor. 10	2,219	1,130	50.92	50	.76
$\frac{C\ K}{c\ k}$	Ab. 10 / Nor. 10	$\frac{C\ K}{c\ k}$	Nor. 10	1,448	1,177	81.28	75	30.50
$\frac{C\ k}{c\ k}$	Nor. 10	Self		6,445	4,771	74.03	75	3.26

of pollen carrying the dominant *C* allele, or to the expected 81.94 per cent calculated when the types of pollen are assumed to be functioning at random.

The data in line 4, table 5, were obtained from the reciprocal of the cross of that which furnished the data of line 1. The percentage of colored seed approximates closely the 50 per cent expected and definitely indicates that the presence of the knob on chromosome 9 does not exert any appreciable influence on the type of pollen that effects fertilization.

A final check on the normal segregation of the dominant *C* allele when paired with the recessive *c* allele, and both alleles are on the knobless type of chromosome 9 is given in line 6, table 5. The observed 74.03 per cent colored seeds is close to the expected 75 per cent.

The foregoing data have demonstrated an abnormal segregation at megasporogenesis of the dominant allele *C* when on the knobbed type of chromosome 9 and paired with the recessive allele borne on the knobless type, but only when the two types of chromosome 10 also are segregating. They also have demonstrated that all classes of pollen from plants heterozygous for the *C c* al-

leles, the knobbed and knobless type of 9, and the two types of 10 effect fertilization in approximately equal numbers.

The data in table 6 combine the preferential segregation at megasporogenesis of the recessive *r* allele borne on the abnormal type of chromosome 10 and the associated preferential segregation of the dominant *C* allele on chromosome 9, caused by the presence of a knob terminating the short arm. It must be remembered that these two preferential segregations work against each other in the production of colored seed. However, the data in tables 3 and 5 show that the tendency to reduce is greater than that to increase the number of colored seeds, consequently there should be a deficiency of colored seeds in selfed progenies in which both preferential segregations occur. The effect of the more pronounced preferential segregation of the abnormal type of chromosome 10 is

TABLE 6

Segregation of the dominant and recessive C c and R r alleles.

PARENTS ♀		PARENTS ♂		TOTAL SEEDS NO.	COLORED SEEDS NO.	PERCENTAGES OF COLORED SEEDS			χ^2	
						OBSERVED	EXPECTED			
							PREFERENTIAL SEGREGATION	RANDOM SEGREGATION	PREFERENTIAL	RANDOM
$\frac{C\ K}{c\ k}$	*r* Ab. 10 / *R* Nor. 10	Self		48,735	25,758	52.85	53.66	56.25	12.76	Highly sig.
$\frac{C\ k}{c\ k}$	*r* Nor. 10 / *R* Nor. 10	Self		13,655	7,609	55.72	—	56.25	—	1.53
$\frac{C\ K}{c\ k}$	*r* Ab. 10 / *R* Nor. 10	*c k*	*r* Ab. 10 / *R* Nor. 10	17,121	7,233	42.25	41.83	37.5	1.22	Highly sig.
c k	*r* Ab. 10 / *R* Nor. 10	$\frac{C\ K}{c\ k}$	*r* Ab. 10 / *R* Nor. 10	11,399	3,736	32.77	32.75	37.5	.003	Highly sig.

illustrated by the data in line 1. The observed 52.85 per cent colored seeds is appreciably below the 56.25 per cent expected from random segregation, but it approaches the 53.66 per cent calculated when preferential segregations at megasporogenesis of 63.87 per cent and 69.01 per cent for the dominant allele *C* and the recessive allele *r*, respectively, are combined with pollen in which all classes are functioning at random.

Line 2 of table 6 is check material in which the observed 55.72 per cent colored seeds is close to the 56.25 per cent expected from a random segregation of these two factors contributing to seed color.

The data in lines 3 and 4 of table 6 are less valuable for measuring the preferential segregation of these two pairs of contrasting alleles. The abnormal type of chromosome 10 and the associated recessive *r* allele are present in the male and female parents in both sets of data, but the absence of a dominant *C* allele in the female parent of one set and the male parent of the other should give 37.5 per cent colored seed if the segregations all were random. The combined effect of preferential segregation of the dominant allele *C* and the recessive allele *r* gave 42.25 per cent colored seeds, a percentage considerably above 37.5

and somewhat higher than the 41.83 per cent calculated from the known preferential segregation. The data in line 4 are a measure of the preferential segregation of the recessive *r* allele only. The observed 32.77 per cent colored seeds is appreciably below that expected from random segregation and is close to the 32.75 per cent expected from a preferential segregation at megasporogenesis of 69.01 per cent. These data show some of the aberrant ratios that are produced when two preferential segregations are taking place simultaneously.

Segregation of the paired *Sh sh* genes

Shrunken, a seed character, is controlled by a gene on chromosome 9. There is about 26 per cent recombination between *sh* and the end of the short arm. Segregation of the contrasting *Sh sh* alleles was determined in heterozygous plants where the *sh* allele was on a knobless chromosome 9.

TABLE 7

Segregation of the dominant and recessive Sh sh alleles.

PARENTS			TOTAL SEEDS NUMBER	*Sh* SEEDS		χ^2
♀		♂		NUMBER	PERCENTAGE	
$\frac{Sh\ K}{sh\ k}$	Ab. 10 / Nor. 10	*sh k* Nor. 10	2,272	1,493	65.71	Highly sig.
$\frac{Sh\ K}{sh\ k}$	Ab. 10 / Nor. 10	Self	34,443	28,350	82.31	Highly sig.
$\frac{Sh\ K}{sh\ k}$	Nor. 10	*sh k* Nor. 10	1,210	606	50.08	.003
$\frac{Sh\ K}{sh\ k}$	Nor. 10	Self	24,634	18,756	76.14	17.04

The data in table 7 show the recovery of the dominant *Sh* allele in progenies in which abnormal 10 was segregating and in check progenies in which only the normal 10 was present. Again the preferential segregation of *Sh* is distinctly associated with the presence of the abnormal 10 in a heterozygous condition.

That the degree of preferential segregation should be slightly higher than that found for the dominant *C* allele was unexpected, since the shrunken locus is a little farther removed from the knob than the *C* locus. The checks, however, may explain this higher percentage, since they, too, exceeded slightly the 50 and 75 per cents expected from random segregation. Shrunken seeds occasionally are not sharply differentiated in strains having a tendency to have a dent type of kernels and so the higher than expected number of nonshrunken seeds in all tests may be due partly to faulty seed classification.

The data on the segregation of the contrasting *Sh sh* alleles, however, are in agreement with the data on the segregation of the contrasting *C c* alleles and show that preferential segregation occurs when contrasting knob types of chromosome 9 and the two types of chromosome 10 are segregating.

Segregation of the paired *Wx wx* genes

A third seed character controlled by genes on chromosome 9 used to test preferential segregation is the *wx* gene, which has about 44 per cent recombination with the end of the short arm. At this distance from a knob it was uncertain if preferential segregation would affect the ratio of waxy to nonwaxy seeds.

Considerable data were accumulated on the segregation of the contrasting *Wx wx* alleles. These data are presented in tables 8 and 9, and in all the progenies tested the recessive *wx* allele was on the knobless or almost knobless type of chromosome 9.

TABLE 8

Segregation of the dominant and recessive Wx wx alleles.

PARENTS ♀		PARENTS ♂	TOTAL SEEDS NUMBER	*Wx* SEEDS NUMBER	*Wx* SEEDS PERCENTAGE	χ^2
$\frac{Wx\ K}{wx\ k}$	Ab. 10 / Nor. 10	*wx k* Nor. 10	24,961	13,336	53.43	Highly sig.
$\frac{Wx\ K}{wx\ k}$	Ab. 10 / Nor. 10	Self	117,450	90,684	77.21	Highly sig.
$\frac{Wx\ k}{wx\ k}$	Ab. 10 / Nor. 10	*wx k* Nor. 10	1,079	521	48.29	1.27
$\frac{Wx\ k}{wx\ k}$	Ab. 10 / Nor. 10	Self	13,949	70,454	75.09	.07
$\frac{Wx\ K}{wx\ k}$	Nor. 10	$\frac{Wx\ K}{wx\ k}$ Nor. 10	21,677	10,927	50.41	1.45
$\frac{Wx\ K}{wx\ k}$	Nor. 10	Self	23,272	17,358	74.59	2.11
$\frac{Wx\ k}{wx\ k}$	Nor. 10	*wx k* Nor. 10	15,792	8,055	51.01	6.40
$\frac{Wx\ k}{wx\ k}$	Nor. 10	Self	35,136	26,399	75.13	.34

Data on four different combinations of the dominant *Wx* allele, both backcrossed and selfed progenies, are reported in table 8. Two of these have the *Wx* allele on a knobbed type and two on a knobless type of chromosome 9. In one of each of these two combinations the *Wx* allele was in plants segregating for abnormal 10 and in the other it was in plants with only the normal type of 10. Only one of these four combinations would be expected, on the basis of the preceding data, to show preferential segregation. In lines 1 and 2 are given the data on the backcross and selfed progenies of this combination, and they both depart from the 50 and 75 per cent expected from random segregation. The data in lines 3 and 4 are the first presented which suggest that the preferential segregation of the abnormal type of chromosome 10 does not influence the segregation of contrasting alleles if these are on paired knobless chromosomes.

The data in table 8 show little to support but nothing to contradict the presence of a preferential segregation at megasporogenesis of a chromosome bearing a knob over the knobless type when abnormal 10 also is segregating.

The data in table 9 are from two tests of pollen behavior when the dominant *Wx* allele is on a chromosome bearing a knob and the recessive *wx* allele is on a knobless chromosome. The percentages of nonwaxy seeds in both tests are about equal, although in one test the two types of chromosome 10 are segregating. Both tests show an appreciable departure from the 50 per cent expected from random segregation; however, such departures are not unusual in segregations of waxy and nonwaxy pollen, and consequently the departure observed

TABLE 9

Segregation of the dominant and recessive Wx wx alleles.

PARENTS			TOTAL SEEDS NUMBER	*Wx* SEEDS		χ^2
♀	♂			NUMBER	PERCENTAGE	
wx k No. 10	$\frac{Wx\ K}{wx\ k}$	$\frac{\text{Ab. 10}}{\text{Nor. 10}}$	2,835	1,549	54.64	24.4
wx k Nor. 10	$\frac{Wx\ K}{wx\ k}$	Nor. 10	2,515	1,361	54.21	17.04

when abnormal 10 was present loses its significance as far as preferential segregation and selective viability is concerned.

Segregation of the paired *Py py* genes

Chromosome 6 is known to have as many as three knobs in some strains of maize. The seedling character known as pigmy controlled by genes on chromosome 6 has lent itself to this study of aberrant segregation. Data from this study of the segregation of the contrasting *Py* and *py* alleles gave the first clue to the effect of the abnormal type of chromosome 10 on the segregation of other chromosomes heterozygous for knobs. Preferential segregation of the abnormal type of chromosome 10 caused preferential segregation of contrasting alleles on chromosome 6 when one of the segregating pair was almost knobless and the other a much knobbed type. This same knobbed and knobless pair of chromosome 6 failed to show preferential segregation when chromosome 10 was normal. These and other observations are shown by the data of tables 10 and 11.

The preferential segregation at megasporogenesis shown in line 1, table 10, is almost as great as that shown in table 3 for the recessive *r* allele. This preferential segregation of the dominant *Py* allele is due to the presence of the abnormal 10, as is apparent when these data are compared with those in line 2, in which the same types of chromosome 6 were present, but abnormal 10 was absent. The data in lines 3 and 4 serve only to further substantiate the fact that only when abnormal 10 is present is there preferential segregation of other homologous chromosomes heterozygous for knobs.

TABLE 10

Segregation of the dominant and recessive Py py alleles.

PARENTS ♀		PARENTS ♂		TOTAL SEEDLINGS NUMBER	*Py* SEEDLINGS NUMBER	*Py* SEEDLINGS PERCENTAGE	χ^2
$\frac{Py\,K\,K(S)\,K}{py\,k\,K(S)\,K(S)}$	$\frac{\text{Ab. 10}}{\text{Nor. 10}}$	$py\,k\,K(S)\,K\,(S)$	Nor. 10	7,278	4,891	67.20	Highly sig.
$\frac{Py\,K\,K(S)\,K}{py\,k\,K(S)\,K(S)}$	Nor. 10	$py\,k\,K(S)\,K(S)$	Nor. 10	2,647	1,301	49.15	.77
$\frac{Py\,k\,k\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	$py\,k\,K(S)\,K(S)$	Nor. 10	4,027	1,967	48.85	2.15
$\frac{Py\,k\,k\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	$py\,k\,K(S)\,K(S)$	Nor. 10	704	348	49.43	.09
$py\,k\,K(S)\,K(S)$	Nor. 10	$\frac{Py\,k\,K\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	132	66	50	.00
$py\,k\,K(S)\,K(S)$	Nor. 10	$\frac{Py\,k\,k\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	610	303	49.67	.03

The last two lines of table 10 give some meager data on the character of the pollen that effects fertilization when the contrasting alleles *Py* and *py*, associated with slight differences in the number and character of the knobs of the two segregating chromosomes, are present at microsporogenesis. Both sets of data show that approximately equal numbers of the different types of pollen effect fertilization of the eggs of the homozygous recessive parent.

TABLE 11

Segregation of the dominant and recessive Py py alleles.

PARENTS ♀		PARENTS ♂		TOTAL SEEDLINGS NUMBER	*Py* SEEDLINGS NUMBER	*Py* SEEDLINGS PERCENTAGE	χ^2
$\frac{Py\,K\,K(S)\,K}{py\,k\,K(S)\,K(S)}$	$\frac{\text{Ab. 10}}{\text{Nor. 10}}$	Self		9,366	7,717	82.39	Highly sig.
$\frac{Py\,K\,K(S)\,K}{py\,k\,K(S)\,K(S)}$	Nor. 10	Self		4,574	3,323	72.65	13.48
$\frac{Py\,k\,K\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	Self		11,466	8,438	73.59	12.13
$\frac{Py\,k\,k\,k}{py\,k\,K(S)\,K(S)}$	Nor. 10	Self		5,577	4,277	76.97	11.46
$\frac{Py\,k\,K(S)\,K(S)}{py\,k\,K(S)\,K(S)}$	Nor. 10	$\frac{Py\,K\,K(S)\,K}{py\,k\,K(S)\,K(S)}$	$\frac{\text{Ab. 10}}{\text{Nor. 10}}$	579	448	77.37	1.74
$\frac{Py\,k\,K(S)\,K(S)}{py\,k\,K(S)\,K(S)}$	Nor. 10	Self		861	656	76.19	.65

Other tests of the segregation of the contrasting *Py py* alleles are reported in table 11. The data in the first four lines are from selfed plants of the same progeny that were used in producing the first backcross plants, the data from which are reported in the first four lines of table 10. They serve principally to confirm the observations from backcross progenies. The data in line 1, however, do give an indirect test of the pollen from plants heterozygous for the two types of chromosome 6. This pollen is functioning at random for the *Py* and *py* types, since the 82.39 per cent of *Py* seedlings is close to the 83.60 per cent expected when a 67.20 per cent preferential segregation at megasporogenesis shown in line 1 of table 10 is combined with an assumed 50:50 ratio of *Py* and *py* types of pollen effecting fertilization.

In spite of the rather extensive data on the segregation of the contrasting *Py* and *py* alleles, they are not sufficient to determine which of the three knobs is most effective in bringing about preferential segregation of those alleles. The central knob may exert little or no influence according to the data of the first line of tables 10 and 11, since preferential segregation occurs when the F_1 plants were homozygous for this particular knob. Only when either the outer or inner knob of chromosome 6 is heterozygous and the other homozygous in a plant segregating for the two types of chromosome 10 will the segregation of the *Py py* alleles show which of these two knobs is nearest the pigmy locus.

False linkage between nonhomologous chromosomes

The data showing that preferential segregation of the two types of chromosome 10 causes a preferential segregation of the knobbed type of chromosomes 9 and 6 over the knobless or almost knobless type also might be interpreted as showing that the recessive *r* allele of the abnormal type of chromosome 10 appears to be linked with the *C*, *Sh*, and *Wx* alleles of chromosome 9 and *Py* allele of chromosome 6. Such apparent linkage is designated as "false linkage," as the term "linkage" usually is restricted to the association of genes in inheritance which results from their being located on the same chromosome. Data showing the false linkage between the *Py* and *C* and the contrasting *py* and *c* alleles that results from preferential segregation are shown in table 12. This false linkage with 41.51 per cent of recombination closely approximates that expected when the preferential segregation for *Py*, *C*, and *r* is 67.2 per cent, 63.87 per cent, and 69.01 per cent, respectively. In contrast, the data from a similar progeny in

TABLE 12

False linkage between the dominant allele Py and the dominant allele C.

F_1 GENOTYPE			LINKAGE PHASE	NUMBERS OF INDIVIDUALS					RECOMBINATIONS			χ^2
				Py C	*Py c*	*py C*	*py c*	TOTAL	NUMBER	PERCENTAGE OBSERVED	PERCENTAGE EXPECTED	
Py K K(S) *K* / *py k K*(S) *K*(S)	*C K* / *c k*	Ab. 10 / Nor. 10	C B	1,119	461	488	218	2,286	949	41.51	42.36	.66
Py K K(S) *K* / *py k K*(S) *K*(S)	*C K* / *c k*	Nor. 10 / Nor. 10	C B	731	775	773	749	3,036	1,548	50.99	50	1.19

which there is no preferential segregation of chromosome 10 shows no evidence of association between *Py* and *C* alleles.

DISCUSSION

Preferential segregation at megasporogenesis has been established for maize chromosomes other than chromosome 10, which are heterozygous for a knob or knobs in plants in which the normal and abnormal types of chromosome 10 also are segregating. This preferential segregation was established by both cytological observations and by its effect on the segregation of contrasting alleles at loci not too far removed from the knob locus.

RHOADES (1942) pointed out that the preferential segregation of the abnormal type of chromosome 10 is unique. The effect of its segregation upon the segregation of other chromosomes is even more unusual. The reason for the preferential segregation of abnormal 10 is at present unexplained, but the preferential segregation of other chromosomes is associated with this unexplainable segregation. Preferential segregation depends upon the presence of knobs, since it is absent when both homologues are knobless.

Cytological data suggest that knob segregations at megasporogenesis may be preferential even when chromosome 10 is normal. Such a preferential segregation was not confirmed by genetic data, but may become apparent when more critical tests are available.

The relationship between the preferential segregation of knobs and the presence of the abnormal type of chromosome 10 must have led, in the past, to the accumulation of knobs in certain maize strains. A glance at LONGLEY's (1938) data on the chromosome morphology of strains of Indian corn shows that abnormal 10 was found in four out of 33 strains and that these four are among the six strains with the largest numbers of knobs. This seeming relationship between a large number of knobs and the presence of abnormal 10 is further substantiated by unpublished data on maize strains from Guatemala. The abnormal type of chromosome 10 is quite prevalent in these strains, and all plants with the abnormal type had more than ten knobs on the remaining nine chromosomes.

This study has been confined largely to the effect of preferential segregation on contrasting alleles at loci near a knob locus. It has shown conclusively that preferential segregation of abnormal 10 causes other chromosomes to segregate abnormally at megasporogenesis if the two homologues consist of one knobbed and one knobless chromosome. The resulting aberrant ratios show the expected approach to ratios of random segregation as the distance from the knob locus to the locus of the segregating alleles increases. This was illustrated in RHOADES' (1942) study of the preferential segregation affecting the contrasting *R r* and *G g* alleles and is further substantiated by the data presented for both chromosomes 10 and 9.

The amount of preferential segregation depends upon the proximity of the gene in question to the knob which is segregating preferentially. The deviation from the expected preferential segregation may be used to determine the dis-

tance between the knob locus and the loci of other alleles on the same chromosome.

False linkage between genes in different linkage groups may lead to some confusion. This effect is not pronounced, however, and under the most extreme preferential segregation it will not indicate linkage with less than 40 per cent of recombination.

SUMMARY

Cytogenetic data have confirmed the studies of RHOADES (1942) on the preferential segregation at megasporogenesis of the abnormal type of chromosome 10. In addition it has been shown that when this preferential segregation occurs in megasporogenesis there will be a preferential segregation of the knobbed over the knobless type of other chromosomes heterozygous for knobs.

The effect of preferential segregation is apparent as aberrant genetic ratios obtained when loci of segregating alleles are near the locus of the segregating knob. Furthermore, the amount of aberrrance may be used as a measure of the distance between a knob locus and the locus of segregating alleles.

Preferential segregation may result in the associated inheritance of genes located on different chromosomes. This association is termed "false linkage."

Since preferential segregation of the abnormal type of chromosome 10 tends to concentrate knob-bearing chromosomes in the megaspore, it may be predicted that abnormal chromosome 10 generally will be found in maize strains with a large number of knobs.

LITERATURE CITED

LONGLEY, A. E., 1938 Chromosomes of maize from North American Indians. J. Agric. Res. 56: 177–195.

RHOADES, M. M., 1942 Preferential segregation in maize. Genetics 27: 395–407.

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